HYPOTHALAMIC-PITUITARY RELATIONSHIPS IN POLYCYSTIC OVARY SYNDROME

by

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Dissertation submitted for the Degree of
Doctor of Medicine
University of Edinburgh 1986

PREFACE

Polycystic ovary syndrome is a common and distressing condition associated with infrequent or absent menstruation and often hirsutism. The cause is unknown but it seems that there is an imbalance between the hormones in the brain, in particular luteinising hormone (LH), and the response of the ovary. Some evidence suggests that there is a deficiency of dopamine (DA) in an area of the brain called the hypothalamus resulting indirectly in excess secretion of LH and that this may be the fundamental problem in PCOS. This evidence is based on two observations. Firstly that the hormone prolactin is increased slightly in some patients with PCOS and secondly that the dopamine agonist bromocriptine may reduce LH concentrations and have a beneficial therapeutic effect. The aim of this thesis was to examine this evidence in detail. Sixty-nine patients with PCOS were involved in the studies. Basal prolactin secretion was evaluated taking account of its complicated physiological variations. Also the response to several agents which stimulate prolactin release was measured. To assess the effect of bromocriptine, a double blind controlled trial was conducted. The possible effect on the complex pulsatile nature of LH secretion was investigated during this trial. The results showed that prolactin secretion was no different from that of normal women and that previous observations of abnormality may have been due to a failure to take adequate account of the normal

variations of spontaneous prolactin secretion or the preceding ovarian function. The controlled trial did not confirm the previously reported beneficial effect of bromocriptine in PCOS. In addition LH secretion was not altered by bromocriptine. However a new approach to the analysis of the pulsatile patterns of LH release suggests that the frequency of pulses is increased in PCOS. Physiologically this can only be explained by an abnormality in the hypothalamus. Therefore, although this study has not confirmed an abnormality of dopamine in PCOS, it offers further evidence in support of a hypothalamic problem in this disease. Further investigation should therefore be directed at this site.

The studies described in this thesis were conducted at the Royal Victoria Infirmary, Newcastle upon Tyne between 1982 and 1985. I am most grateful to Professor Pat Kendall-Taylor for her ideas and advice in the initial planning of the work and for her continuing support during the study. Particular thanks are also due to Professor Bill Dunlop for his encouragement and patience, and for his invaluable advice with the presentation of the results. The excellent technical advice and assistance of Mrs Linda Brown with the assays is acknowledged and the nursing skills of Sister Esme Harris and Sister Mavis Harris and their staff were greatly appreciated by patients and volunteers. For the statistical

analysis of serial data by time series analysis, the expert assistance of Dr Peter Diggle, Department of Statistics, University of Newcastle upon Tyne, was gratefully received. Mr Ken MacClean of the Department of Medical Photography took the photographs in the hirsutism study and thereby assured their high standard. Finally the co-operation of the patients and normal volunteers who agreed to participate in this study is acknowledged since this thesis would not have been possible without their help.

The work reported here has been described in the following publications:

Murdoch AP, Dunlop W, Kendall-Taylor P, Watson MJ (1984)
The acute effects of a dopamine antagonist (Domperidone) on luteinising hormone, follicle stimulating hormone, prolactin and thyrotrophin secretion in polycystic ovary syndrome: diifferential effect of ovulation.
Clinical Endocrinology 21; 611-619.

Murdoch AP, Diggle PJ, Dunlop W, Kendall-Taylor P (1985)
Determination of the frequency of pulsatile luteinising hormone by time series analysis.
Clinical Endocrinology 22: 341-346.

Murdoch AP, Dunlop W, Kendall-Taylor P (1986)
Studies of prolactin secretion in polycystic ovary syndrome.
Clinical Endocrinology 24: 165-175.

Murdoch AP, MacClean K, Kendall-Taylor P, Dunlop W (1986)
The treatment of hirsutism in polycystic ovary syndrome with bromocriptine.
British Journal of Obstetrics and Gynaecology. In press.

This thesis has been composed by myself and is my own work.

CONTENTS

CHAPTER 1: INTRODUCTION

Physicians of the utmost fame
Were called at once, but when they came
They answered, as they took their fees
'There is no cure for this disease.'

(Belloc)

Sadly, such an answer awaits those who suffer from polycystic ovary syndrome (PCOS). Despite a century of recognition, there is still no cure and this reflects a failure to understand the basic pathology. Ovarian function is clearly disordered in PCOS although the precise mechanism of this dysfunction is unknown. Control of the ovary involves a complex and delicate interplay between its own hormones and those of the brain and it is this endocrine system that is abnormal in PCOS.In this study an attempt has been made to test an hypothesis related to one possible endocrine abnormality in PCOS. Before this hypothesis can be understood an explanation of normal ovarian regulation is needed.

1.1 REGULATION OF FEMALE REPRODUCTION

1.1.1 Historical background

The mysteries of reproduction have baffled scientists for centuries. Each new discovery seems to have revealed only more complex questions. The earliest studies of the growing embryo were recorded by Hippocrates. It was not until the 17th century though and the development of the microscope, that the origins of the embryo were revealed. Initial findings were misleading. Hamm and Leeuwenhoek observed the sperm for the first time in 1677 and thought they saw within it a tiny but complete human. The presumed role for the female was thus simply to provide the womb to nurture this growing fetus. A century passed before Spallanzani showed in 1775 that both sperm and egg were required for an embryo to develop.

The last 150 years however have seen the most rapid advances in the understanding of reproduction. The cell theory, stating that the body is composed of cells and cell products, was established in 1839 by Schieden and Schwann and the discovery of chromosomes and the principles of genetic inheritance began to be uncovered. The interaction between the egg, sperm and their genes was established as the centre of the reproductive process. However, it became clear that there were hormones which regulate the egg's development which were of equal importance in reproduction.

The ovary was known to be the source of the egg but workers in the 19th century showed that it also had other functions. The

existence of ovarian hormones was implied from the results of experimental removal of the ovaries. This operation was invariably followed by atrophy of the genital tract. Although early workers attributed these findings to incidental causes such as interference with the vasular supply (Hofmeier 1880) or nervous connections (Sokoloff 1896) of the uterus, it was later established that the ovary produced hormones and it was the removal of these factors which caused genital regression. In the late 19th century, isolated extracts of the ovary were used successfully to reverse the atrophy of the genital tract in women without ovaries thus confirming the existence of ovarian hormones. However, it was much later that the pure oestrogen, oestriol, and then the progesterone metabolite, pregnanediol, were purified from urine (Marrian 1929). Extraction of the active hormones from the ovary proved more difficult and numerous substances with differing biological activity were found. Two steroid compounds, oestradiol-17B (McCorquodale et al 1935) and progesterone (Wintersteiner and Allen 1934), were the most potent ovarian factors isolated. Subsequent studies established that these are the main steroid hormones secreted from the ovary.

More recently a peptide hormone, inhibin, has been isolated from the ovarian follicle. It is undoubtedly an important regulator of gonadotrophins although its physiological role is still being investigated (Chappel et al 1980).

The role of the brain in controlling reproduction was illustrated in 1936 by Marshall and Verney who showed that

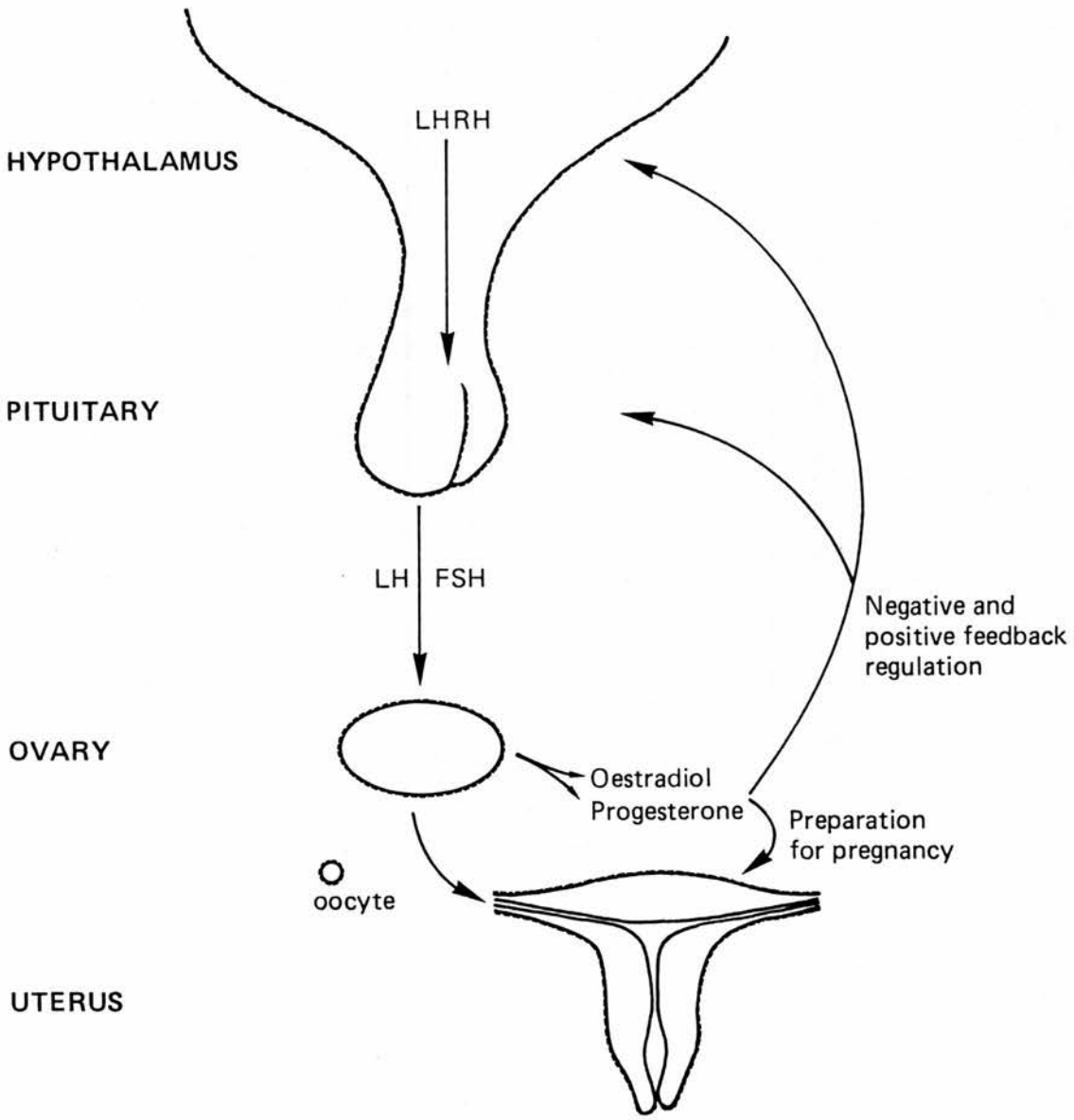
electrical stimulation of the rabbit brain resulted in ovulation and pseudopregnancy. Numerous studies using more localised stimulii subsequently revealed that two specific areas of the brain were primarily involved; the hypothalamus and the pituitary gland. Anatomical studies showed that these areas were closely linked by nerve fibres in the pituitary stalk and the surrounding blood vessels. The importance of this link in reproduction was reportedly demonstrated by Hinsey in 1937. In these studies, transection of the pituitary stalk in rabbits resulted in atrophy of the ovary. Again more specific studies subsequently showed that, for reproduction, the important part of the link was the blood vessels around the stalk. A humoral substance was postulated which could be released from the hypothalamus by electrical stimulation, secreted into the portal vessels and thence carried to the pituitary gland. This hypothesis was proved when the hormone was eventually isolated and its structure determined in 1971 (Matsuo et al). It was given the name luteinising hormone releasing hormone (LHRH) and, as postulated, its principle action was found to be on the pituitary gland.

The existence of pituitary factors involved in regulation of reproduction was suggested in early experiments in which removal of the dog pituitary gland caused atrophy of the testes. Further evidence was obtained by Long and Evans (1922) who showed that ox pituitary extracts caused luteinization of the ovaries of rats. The source of these hormones (gonadotrophins) was identified as the anterior part of the pituitary by Smith and Engle in 1927. Then Fevold et al reported in 1931 that they had separated two

anterior pituitary substances which had different actions on the ovary; one promoted follicle development and the other luteinisation. The names follicular stimulating hormone (FSH) and luteinising hormone (LH) were thus applied. The development of methods of radioimmunoassay in the last 20 years enabled specific measurement of these hormones. Their precise role in the control of the ovary in women has subsequently been studied in detail.

A complex interaction has been demonstrated between the ovarian hormones oestradiol, progesterone and inhibin, the pituitary hormones LH and FSH and the hypothalamic hormone LHRH (Figure 1.1). The evidence for these links, on which our current understanding of ovarian control is based, is described in more detail in the following sections.

FIGURE 1.1
REPRODUCTION CONTROL PATHWAYS IN THE FEMALE



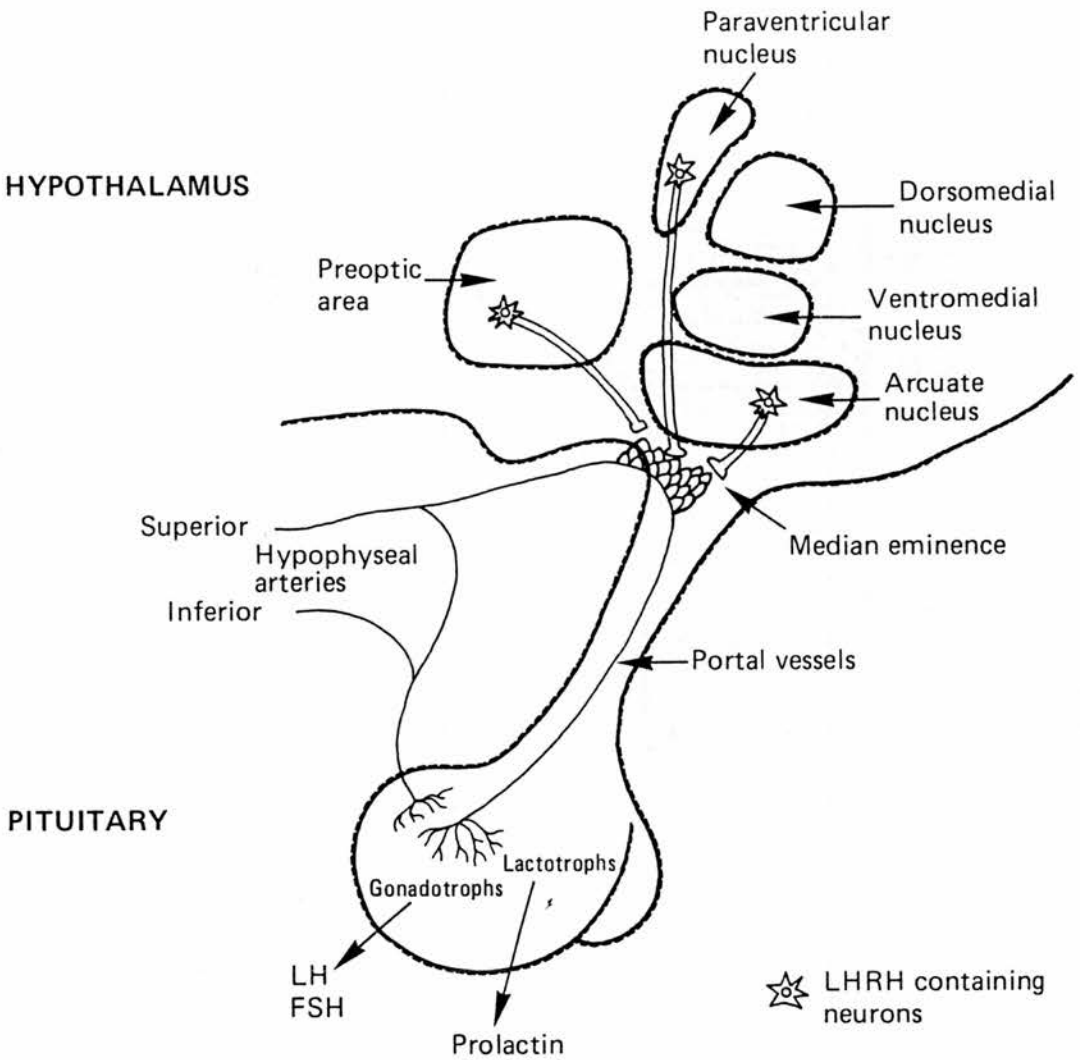
1.1.2 Anatomy of the hypothalamic - pituitary - ovarian axis

HYPOTHALAMUS

The anatomical relationship between the hypothalamus and pituitary gland is shown in Figure 1.2. The hypothalamus is a small area lying in the midline in the base of the brain. It contains several aggregations of neurons, the hypothalamic nuclei; each being concerned with specific control functions and synthesising its own specific releasing hormone. Neurons from these areas project axons down to the median eminence of the hypothalamus where the nerve terminals then lie close to the loops of the portal vessels. LHRH secreted from these neurons enters the portal veins surrounding the pituitary stalk and passes directly to the pituitary gland. Immunohistochemical techniques using LHRH labelled antibodies in rats have localised the neurons containing LHRH to be principally in the arcuate and para-ventricular nuclei and in the preoptic area (Fuxe et al, 1978). Although not all workers have been able to confirm these observations specifically, the variations may be explained by species differences. The principle of more than one hypothalamic nucleus being involved in LHRH secretion is generally accepted (McCann and Moss 1975).

The blood supply to the part of the hypothalamus principally concerned with LHRH neurons is directly from the systemic circulation. The localisation of LHRH neurons at several sites allows there to be a possible synaptic contact with numerous other hypothalamic neurotransmitters. Animal studies have shown that dopamine (DA) is found widely in the central nervous system

FIGURE 1.2
HYPOTHALAMIC PITUITARY RELATIONSHIPS



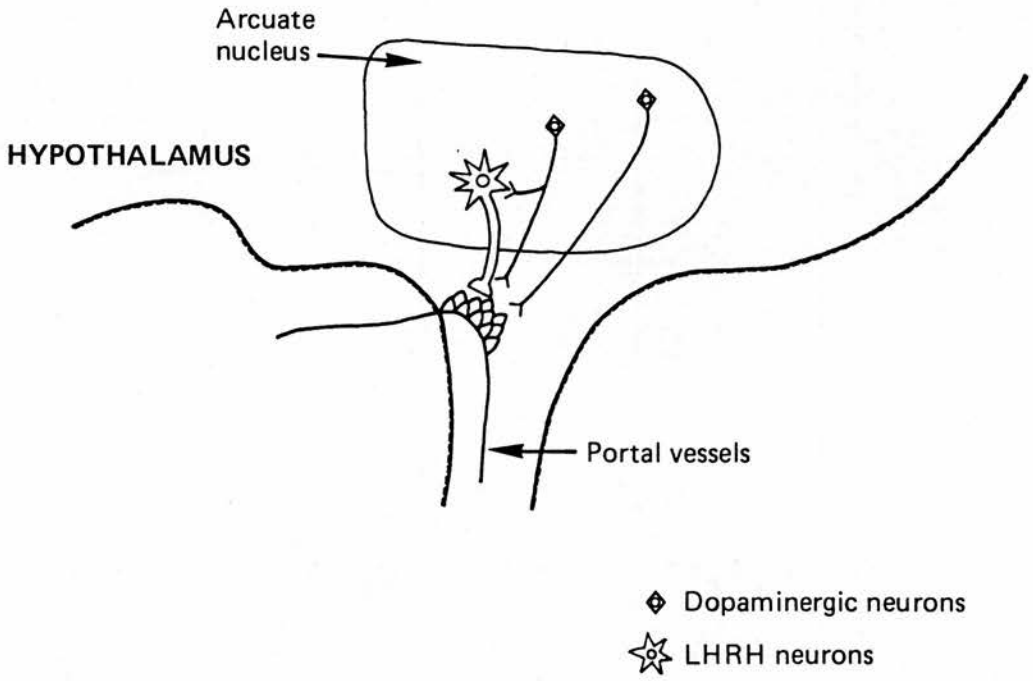
but there are several collections of dopaminergic neurons within the hypothalamus (Fuxe 1965, Fuxe et al 1978, Bjorkland et al 1973). In particular a cluster can be found in the arcuate nucleus (Figure 1.3). Some of the nerve terminals from these nuclei end at the portal vessels whilst others appear to synapse with LHRH nerve terminals. LHRH neurons are also closely associated with serotoninergic terminals (Fuxe and Hokfelt 1969) and histaminergic neurons (Brownstein et al 1973) are found in the median eminence. Since cholinergic terminals are found widespread in the hypothalamus (Schute 1970), acetylcholine may also be involved in LHRH regulation. The endorphins are found in the arcuate nucleus and median eminence in close association with LHRH neurons (Yen 1980b). Whilst anatomical links do not necessarily imply functional relationships, all these various neurotransmitters may be involved in LHRH regulation. One which has been more extensively investigated than most is dopamine. This is discussed in detail in Section 1.1.3.

PITUITARY GLAND

The pituitary gland is small, weighing only 500 mg. Within the anterior part of the gland are several cell types. In particular, there are basophilic cells containing LH and FSH (gonadotrophs). Synthesis and secretion of LH and FSH occurs from these cells (Figure 1.2). As well as receiving a blood supply via the portal veins, a rich supply from the systemic circulation also reaches the anterior pituitary gland via the superior and inferior hypophyseal branches of the internal carotid artery. The venous

FIGURE 1.3

DOPAMINERGIC NEURONS IN THE ARCUATE NUCLEUS: POSSIBLE RELATIONSHIP WITH LHRH NEURONS



drainage which contains the secreted pituitary hormones is to the systemic circulation.

OVARY

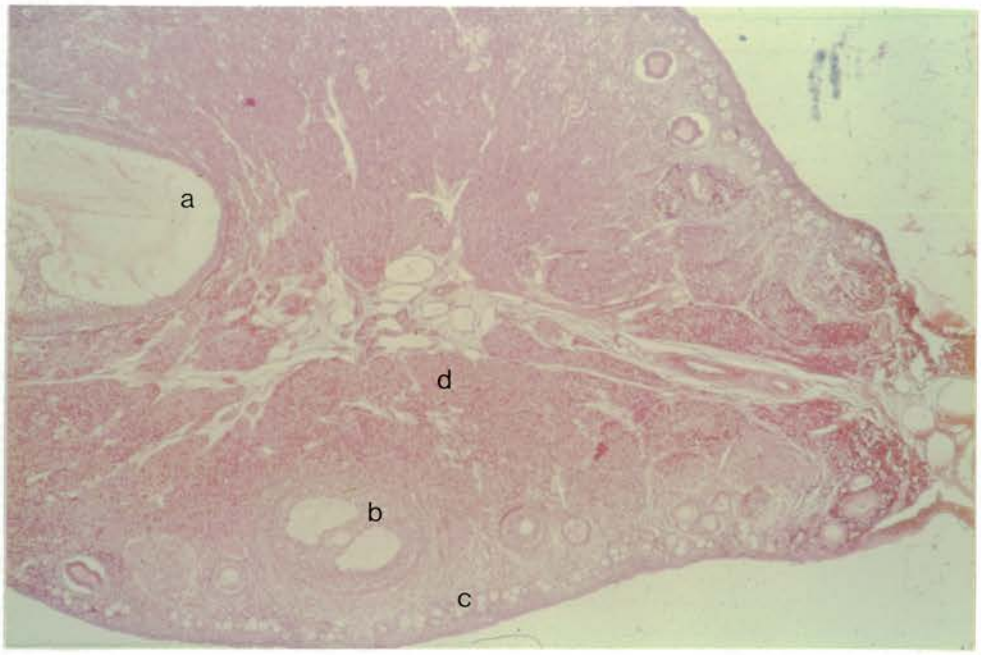
A normal ovary is shown in Figure 1.4. It is found within the abdominal cavity and, in the adult, is about 2 x 3 cm in size. Histologically it consists of stromal tissue containing primordial follicles surrounded by glandular tissue. These primordial follicles are formed in neonatal life and, under appropriate gonadotrophin stimulation, these follicles mature. Each contains a primary germ cell or oocyte. Surrounding the oocyte is a layer of granulosa cells, a basement membrane and a theca cell layer outside the basement membrane. The interactions between steroid secretion from the granulosa and theca cells is crucial in both follicular development and in feedback control of the gonadotrophins. The details of steroid secretion are discussed in more detail later.

In the regulation of the ovarian cycle, the hypothalamus, pituitary and ovarian components cannot be separated. Nonetheless for clarity the physiological relationships will be discussed individually in the following sections.

Figure 1.4 NORMAL OVARY

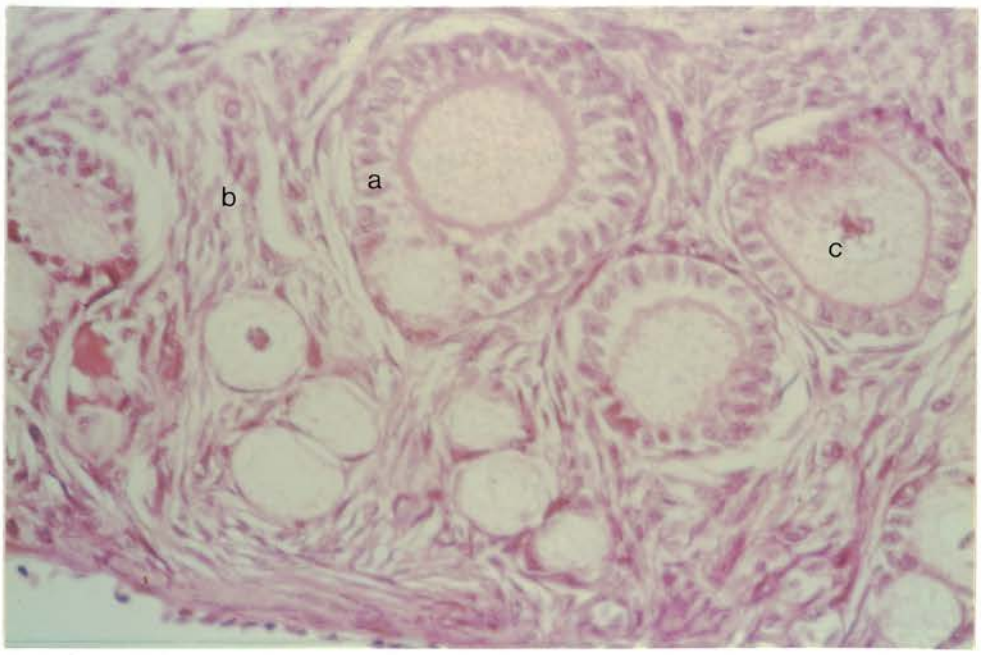
CROSS SECTION OF OVARY

a. Mature follicle
b. Immature follicle
c. Primordial follicles
d. Stroma



DEVELOPING FOLLICLES

a. Granulosa cells
b. Theca cells
c. Oocyte



1.1.3 Hypothalamic regulation of gonadotrophin secretion

There is a general acceptance that LHRH is the principle hypothalamic regulator of LH and FSH synthesis and secretion. Neutralisation of the endogenous LHRH in monkeys using specific antibodies results in the decline of gonadotrophin release (McCormack et al 1977). The prompt initiation of the ovarian cycle using exogenous LHRH in women with primary deficiency of LHRH is an indication of the importance of LHRH in the human ovarian cycle (Crowley and McArthur 1980).

ACTIONS OF LHRH

LHRH is a decapeptide, [(Pyro)-Glu-His-Tryp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2]. The availability of a synthetic analogue has enabled the physiological role of LHRH to be studied in detail. In humans it causes a release of both LH and FSH (Kastin et al 1972, Yen et al 1972). More detailed studies using varying amounts of LHRH identified that small doses increased the pool of LH stored in the gonadotroph without initiating release whereas large doses cause release of LH (Hoff et al 1979). These priming and releasing actions of LHRH are dissociable and thus may be part of separate control mechanisms. Further evidence to support this theory is the differing response of the two pools of LH to oestradiol. The latter tends to inhibit the releasing action of LHRH whilst promoting synthesis (Lasley et al 1976, Wang et al 1976).

LHRH PULSES

Following the initial observations in rhesus monkeys (Dierschke et al 1970), studies of LH secretion in women found that it is secreted in discrete pulses. These have been shown to occur at intervals between one and four hours (Midgley and Jaffe 1971, Yen et al 1972a) and variations in LH pulse amplitude and frequency have been seen during the ovarian cycle (Santen and Bardin 1973, Reame et al 1984).

The origin of the pulses is generally agreed to be the hypothalamus. Although clearly it is not possible to sample the portal vein in humans, studies have been performed in monkeys. Circhorial oscillations of LHRH were recorded (Carmel et al 1976). More recent investigations using a sheep model demonstrated that each LH pulse was associated with an LHRH discharge (Clarke and Cummins 1982).

The physiological significance of a pulsed LHRH signal is uncertain. A continuous infusion of LHRH in hypophysectomised monkeys results in a dramatic fall in LH which can only be restored by pulsed LHRH administration (Belchetz et al 1978). A possible explanation involves the LHRH receptors on the gonadotroph. Constant supplies of LHRH may cause desensitisation of the target cells by receptor loss and the normal recognition and processing of the LHRH+receptor complex may require alternating periods of rest and activity (Knobil and Plant 1978).

When discussing the hypothalamic regulation of gonadotrophin secretion, therefore, the pulsatile nature of the LHRH signal must be considered. The frequency of the LHRH signal determines the

frequency of the LH pulses. The pituitary response and hence the amplitude of the LH pulses is modulated by the relative sensitivity of the gonadotrophin pools.

DOPAMINE

Of the many hypothalamic neurotransmitters, the one most frequently implicated in LHRH regulation is dopamine (3,4 dihydroxyphenylethylamine). Structurally DA is closely related to another neurotransmitter, noradrenaline. In animal studies there is evidence that these two catecholamines have contradictory effects on LHRH secretion consisting of inhibition by DA and stimulation by noradrenaline (McCann and Moss 1975, Drouva and Gallo 1977, Fuxe et al 1978, Gallo 1978, Anden et al 1970, Negro-Vilar et al 1982). In humans, the evidence for a regulatory role for noradrenaline is less than convincing but the involvement of DA in LHRH control seems more likely.

In normal women DA, infusion has been shown to decrease LH secretion (Martin et al 1981; Judd et al 1978, Judd et al 1979; Pontiroli, 1980) and also suppress spontaneous LH pulses (Martin et al 1981). Similar LH inhibition follows L-Dopa treatment (Lachelin et al 1977). The LH response to DA varies during the menstrual cycle (Judd et al 1978) and can be modulated by oestrogen therapy (Judd et al 1979).

However, DA antagonists metoclopramide, monoiodotyrosine and domperidone have failed to demonstrate an alteration in LH secretion in normal women (Quigley et al 1979; Quigley et al 1980; Anderson et al 1982a; Anderson and Tabor 1982b; Seki et al 1982; Murdoch et al 1984; Ho et al 1984) and in hypogonadal women

LH pulsatility was maintained despite DA receptor blockade (Anderson and Tabor,1982).

A possible explanation for these inconsistent findings is that in none of these studies were the detailed patterns of LH pulsatile secretion determined. DA may modify the pulse patterns of LH release and such changes would not be apparent in short term studies.

In this section it has been shown that the major stimulator of the gonadotroph is the hypothalamic releasing hormone LHRH. This is released in pulses and has both priming and releasing effects on the gonadotroph. In addition the neurotransmittor, DA, has been demonstrated to be involved in the regulation of LHRH secretion. Additional modulating factors of the hypothalamic-pituitary axis are the ovarian steroids oestradiol and progesterone and their role will now be examined further.

1.1.4 Ovarian regulation of gonadotrophin secretion

Although LHRH is essential for the synthesis and secretion of LH and FSH, the cyclical pattern of their release is regulated primarily by the feedback effects of the two ovarian sex steroids, oestradiol and progesterone. The daily changes in these hormones observed during the ovarian cycle are shown in Figure 1.5 (Baird 1980).

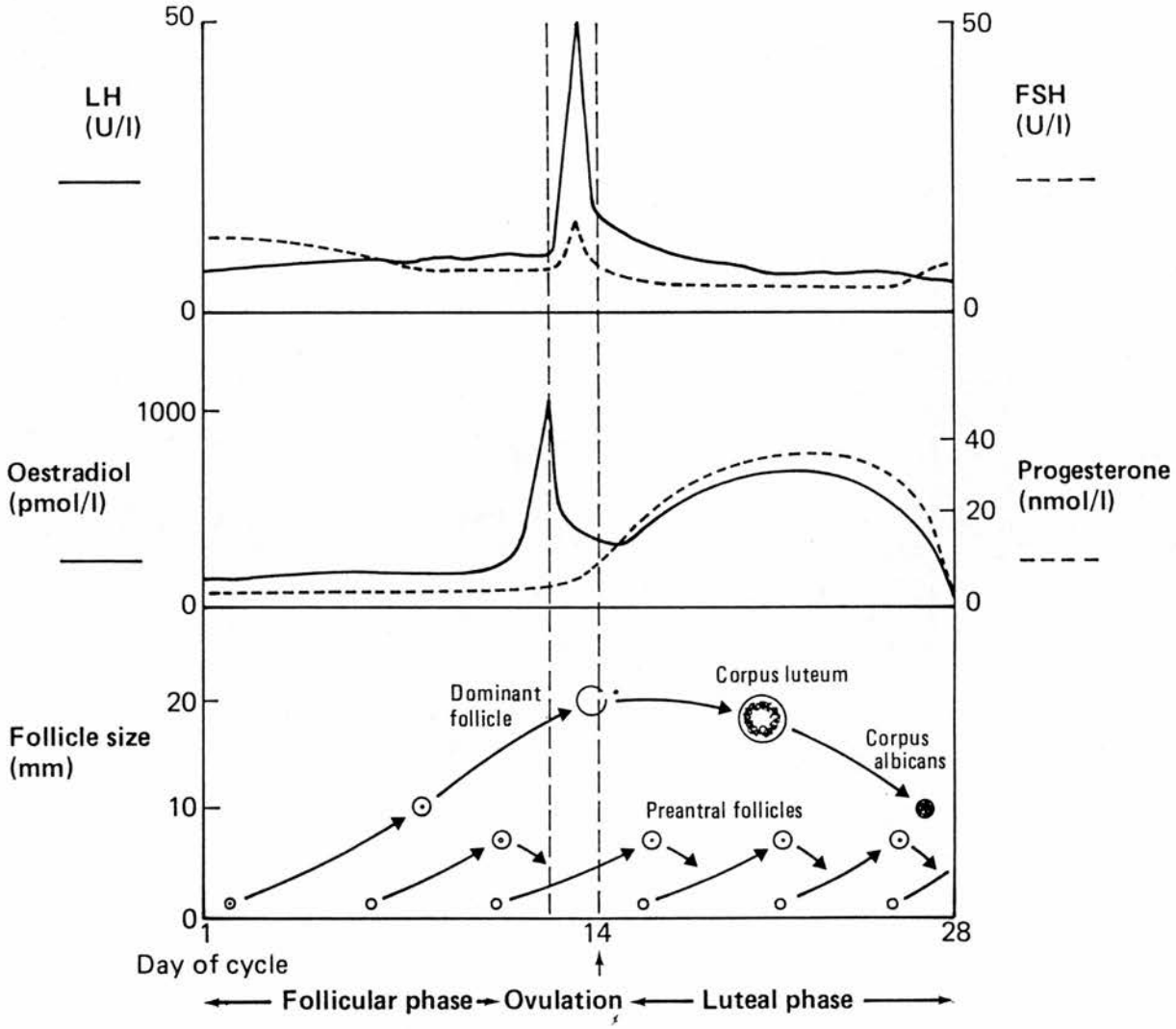
EFFECTS OF OESTRADIOL

Oestradiol can either inhibit (negative effect) or stimulate (positive effect) LH secretion. The negative effect is most clearly illustrated in post-menopausal women who are oestrogen deficient; high gonadotrophin concentrations in these women can be promptly suppressed with oestrogen therapy (Nillius and Wide 1970). The positive feedback effect is illustrated physiologically in the ovarian cycle (Figure 1.5). A sudden rise of oestrogen just before ovulation is followed by a surge of LH. These two feedback effects can further be demonstrated by giving oestrogens exogenously to pre-menopausal women. Initial LH inhibition is seen over the first few hours followed by an LH rise about 48 hours later (Shaw et al 1975b, Baird et al 1977).

MECHANISMS OF OESTRADIOL EFFECTS

The mechanisms by which oestradiol modulates gonadotrophin secretion are uncertain. Studies in rhesus monkeys have shown that after ablation of the arcuate nucleus and the administration of constant LHRH pulses, both positive and negative feedback actions of oestradiol on the gonadotroph are maintained (Knobil et

FIGURE 1.5
GONADOTROPHIN AND OVARIAN CHANGES DURING THE OVARIAN CYCLE



Adapted from Baird (1983)

al 1980). This suggests that oestradiol modulates the gonadotroph directly. In women, studies of the LH response to LHRH during the menstrual cycle showed that the pituitary sensitivity increased with rising plasma oestradiol concentrations (Lasley et al 1975). As explained earlier, this may have reflected changes in the proportions of stored and releasable LH in the gonadotroph which were altered by oestradiol.

A hypothalamic site for oestradiol feedback regulation of LHRH is possible. Oestrogen receptors have been discovered in many parts of the rhesus monkey hypothalamus (Gerlach et al 1976). However, a direct effect of oestradiol on LHRH secretion has not yet been found.

EFFECTS OF PROGESTERONE

By contrast progesterone appears to have its principle action on the hypothalamus. Administration of progesterone to women has been clearly shown to reduce LH pulse frequency (El Sheikh et al 1983, Soules et al 1984) which was presumed to reflect a decreased LHRH pulse frequency. An independent action of progesterone on the gonadotroph has not been found.

Physiologically, the interplay between oestradiol and progesterone in gonadotrophin regulation is probably much more complicated. In high dose it appears to enhance the negative feedback effects of oestrogens whilst blocking the positive effect (Spies and Niswender 1971). By contrast, low doses seem to enhance the positive feedback effects of oestrogens (Liu and Yen 1983).

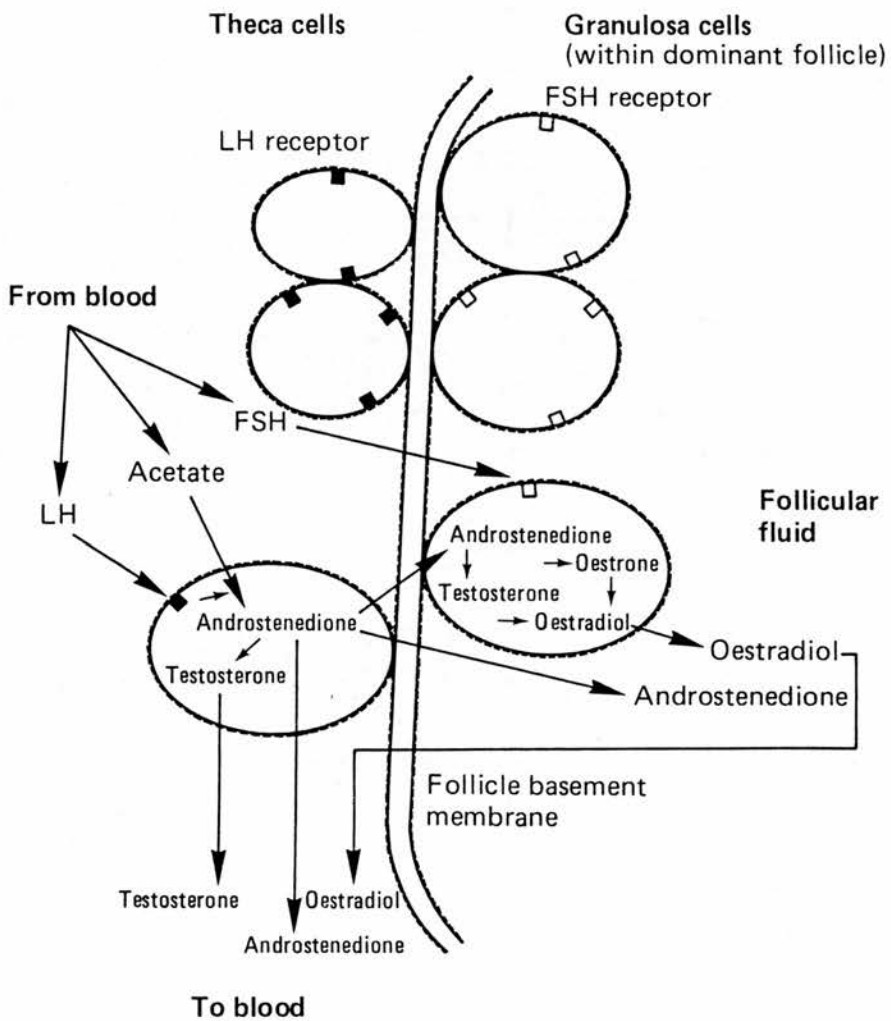
It seems therefore that the sex steroids have a controlling influence on both the hypothalamus and the pituitary gland in regulating gonadotrophin secretion. Hence it follows that abnormalities of steroid secretion may be expected to cause abnormal gonadotrophin concentrations by alteration of both the frequency and amplitude of their pulsatile secretion. The source of these steroids is the ovaries and specific sites of their production will now be described.

1.1.5 Ovarian cycle and steroid secretion

The functional unit in the ovary is the follicle. The egg develops within it and the principle secretions of the ovary originate from cells within and immediately around it. From birth it is thought that a steady supply of primordial follicles enters the active growth cycle until the menopause is reached and that up to 15 follicles may develop each month. With adequate gonadotrophin stimulation these follicles will increase in size (up to 10mm) but most (preantral follicles) will then regress. One in each cycle (the dominant follicle) has been shown to develop until ovulation occurs (Baird 1980). The hormone milieu at the initiation of follicular growth has been considered to be the crucial factor determining the development of this dominant follicle - in particular a small rise in FSH (Figure 1.5) (Midgley and Jaffe 1968). This dominant follicle accounts for most of the ovarian steroid production and this is discussed below in greater detail.

The synthesis and secretion of hormones from the dominant follicle are illustrated in Figure 1.6. Theca cells around the follicle were found to possess LH receptors and under LH stimulation they secreted the androgens, androstenedione and testosterone (Han et al 1974). Androstenedione in particular enters the developing follicles where it is the precursor for further steroid synthesis. In addition the theca cells account for most of the androstenedione circulating in the plasma. A rise in androstenedione coincident with the midcycle LH peak is further

FIGURE 1.6
BIOSYNTHESIS OF STEROIDS WITHIN THE OVARY



evidence to support the relationship between androstenedione and LH in women (Abraham 1974, Judd and Yen 1973, Kim et al 1949).

Granulosa cells lining this follicle develop FSH receptors. Under the influence of FSH they acquire an aromatase enzyme and hence gain the ability to convert androstenedione to oestradiol. The synergistic effect of oestradiol and FSH induces granulosa cell proliferation resulting in further oestradiol secretion (Dorrington and Armstrong 1979). The dominant follicle therefore contains relatively high oestradiol concentrations. The preantral follicles which have not been influenced by the FSH rise in the early stage of the cycle do not acquire aromatase activity and therefore cannot synthesise oestradiol. They thus retain high intrafollicular androstenedione concentrations. It is thought that this balance between androstenedione and oestradiol is crucial for the continued growth of the follicle; low androstenedione relative to oestradiol being a prerequisite for complete development to the preovulatory state (Hillier et al 1980).

Prior to ovulation the dominant follicle develops LH receptors and can therefore respond to the preovulatory surge of LH. This induces release of the egg and subsequent luteinisation of the granulosa cells. The corpus luteum which then forms will, under further stimulation by LH, secrete progesterone (Baird 1980). The observation of high progesterone concentrations in the luteal phase of the cycle is indicative of luteal function. For practical purposes this is considered to indicate that ovulation has occurred.

In summary therefore, some of the factors required for folliculogenesis are: normal LH concentrations to stimulate androstenedione secretion; FSH to stimulate aromatase activity and allow the conversion of androstenedione to oestradiol; and the correct balance of androstenedione and oestradiol to allow the follicle to mature to the preovulatory state.

1.2 PROLACTIN AND REPRODUCTION

In addition to the hormones described in the preceding chapters which are the principal hormones known to regulate human reproduction, another pituitary hormone, prolactin, may also be involved in reproduction. Its place in ovarian control in other species is not disputed but in humans a physiological role, other than during lactation, has not been found. However under pathological conditions as well as during lactation, high prolactin concentrations have a profound inhibitory effect on ovarian function (McNeilly 1980). The links between the regulation of gonadotrophins and prolactin are outlined below.

1.2.1 Hypothalamic regulation of prolactin secretion

DOPAMINE

Prolactin secretion from the anterior pituitary gland differs from that of other pituitary hormones since its release is under constant inhibition by a hypothalamic inhibitory factor. This is generally accepted to be DA since administration of DA (Leblanc et al 1976) or a DA agonist (Lachelin et al 1977) to humans invariably depresses prolactin release. In addition, DA antagonists promote secretion (Judd et al 1976).

DA receptors are found in the pituitary gland and they are predominantly located on the lactotrophs (Goldsmith et al 1979). Neurons in the arcuate nucleus release DA into the portal vessels and the inhibitory effect is thought to be direct at the lactotroph (Ben Jonathan et al 1977). Thus, although the sites of DA neurons affecting LHRH and prolactin secretion are similar, the

targets of action differ. LHRH is inhibited by DA modulation of its release into the portal veins whilst prolactin is inhibited by a direct pituitary action on the lactotroph.

A short loop feedback system is also thought to operate in prolactin regulation. Retrograde blood flow in the hypothalamic-pituitary vascular bed has been demonstrated (Bergland and Page 1978). Prolactin secretion from the lactotroph may therefore pass back to the hypothalamus and regulate DA secretion directly. It would thus follow that hypothalamic DA concentrations would be elevated in association with hyperprolactinaemia.

LHRH

The hypothalamic releasing hormone LHRH, when given in pharmacological doses, stimulated prolactin release. The rise was small and again was of uncertain physiological relevance (Yen et al 1980b, Casper and Yen 1981). Prolactin secretion has been noted to be pulsatile (Ehara et al 1973, Backstrom et al 1982) and synchrony between spontaneous pulses of LH and prolactin have been recorded (Cetel and Yen 1983, Braund et al 1984). This may possibly reflect a common pulse generator such as LHRH.

1.2.2 Ovarian regulation of prolactin secretion

Oestrogens have a stimulatory effect on the lactotroph. In women the oestrogen concentrations vary during the ovarian cycle and prolactin secretion related to these changes has been investigated. During the slow oestrogen rise over the first half of the ovarian cycle there is no change in prolactin concentration but a midcycle peak coincident with the oestrogen peak has been observed (Ehara et al 1973, Aksel 1981, Backstrom et al 1982). Additional evidence for the relationship between oestradiol and prolactin is the effect of prolonged pharmacological doses of oestrogen; prolactin synthesis and release in women was found to be promoted (Yen et al 1974).

Animal studies suggested that oestradiol has a direct pituitary action since it caused lactotroph hyperplasia and hypertrophy (Meites et al 1972, Franks 1983). Although not firmly established, it seems that oestrogens may affect prolactin secretion by modulating DA regulation. In rats, oestrogen receptors are present on the DA neurons in the arcuate nucleus and hypothalamic DA content is inversely related to the oestrogen status (Selmanoff et al 1976, Donoso et al 1967, Eikenberg et al 1977, Fuxe et al 1969). In addition, portal vein DA concentrations may be decreased with oestrogen therapy (Ben Jonathan et al 1977) resulting in hyperprolactinaemia.

1.2.3 Other factors regulating prolactin secretion

Several other factors modulate prolactin secretion. The hypothalamic releasing hormone thyroid releasing hormone (TRH) causes prolactin secretion when given intravenously (Jacobs et al 1971, McNeilly and Hagan 1974) although whether this is of physiological significance is unknown. Prolactin is also released in response to stress and sleep (Copinski et al 1975, Ehara et al 1973). Ingestion of high protein food causes prolactin release, possibly as a result of ingestion of neurotransmitter substrates affecting the hypothalamic control pathways (Ishizuka et al 1983,Quigley et al 1981b). The physiological significance of these various modulating factors of prolactin secretion is unknown but clearly they must be taken into consideration when evaluating lactotroph function.

1.3 RELATIONSHIPS BETWEEN GONADOTROPHIN AND PROLACTIN SECRETION

It has already been shown that there are several factors that have similar effects on both the gonadotroph and the lactotroph. Oestrogens and LHRH stimulate secretion from both whilst DA inhibits LH and prolactin release. Whether these similarities have a physiological significance in women is not known. Nonetheless a functional link between prolactin and gonadotrophins is seen in hyperprolactinaemia both physiological (during lactation) and pathological (pituitary tumours).

Hyperprolactinaemia, from whatever cause, is associated with amenorrhoea. It may result from lactation, pituitary tumour or be drug induced (Delvoye et al 1977, McNeilly 1980). In each case, the resulting ovarian suppression is similar. However, the ovary is not the site of the problem. It remains responsive to gonadotrophins since exogenous therapy was shown to induce ovulation (Leyendecker et al 1980). An abnormality of gonadotroph function was demonstrated in studies of LH secretion in hyperprolactinaemic women. These were characterised by a decrease in LH pulses (Glaiser et al 1984, Klibanski et al 1984) and an impairment of the LH response to LHRH.

Hyperprolactinaemia may be only an associated phenomenon to the gonadotroph dysfunction rather than the cause. However, the evidence suggests that prolactin is more likely to be the culprit. The DA agonist bromocriptine is now used widely in the treatment of hyperprolactinaemia and by reducing prolactin concentration it is highly effective in restoring ovulation regardless of the underlying cause.

The mechanisms by which hyperprolactinaemia alters gonadotroph function are unknown. Studies in hyperprolactinaemic women found an exaggerated fall in LH concentrations after DA receptor blockade. This suggested that tonic LH inhibition by DA may have been increased (Quigley et al 1979). These results taken together with the factors discussed in the preceding sections, offer strong evidence that prolactin and gonadotrophin secretion are linked by complex interactions between LHRH, DA and oestradiol. How this is related to PCOS is explained in the following sections.

1.4 POLYCYSTIC OVARY SYNDROME

1.4.1 Historical background

The history of polycystic ovary syndrome (PCOS) starts with the ovary since it was the anatomical abnormality of the ovary which was first described in 1844 by Chereau. These enlarged pearly white ovaries with thickened capsules were thereafter removed under the belief that they caused a spectrum of gynaecological problems from dysmenorrhoea to menstrually related epilepsy! By the turn of the century a more conservative approach was usually adopted and wedge resection was advocated. The link between the abnormal ovary and the typical clinical symptoms was made in 1935 by Stein and Leventhalto whose names the syndrome was subsequently applied (Stein and Leventhal 1935).

The clinical features that they described were amenorrhoea, obesity, hirsutism and infertility. However, these symptoms are variable. In a later review, Stein reported that many of his patients had oligomenorrhoea rather than amenorrhoea, only 50% were hirsute and the 10% incidence of obesity was not thought to be greater than that of the general population (Stein 1945). Similar findings are reported in later reviews (Yen 1980, Goldzeiher 1981, Futterweit 1984). Given this clinical variation the diagnosis has rested on the observation of the typical appearance of the ovaries.

1.4.2 The polycystic ovary

A typical polycystic ovary is shown in Figure 1.7. Although classically enlarged with a smooth capsule and underlying multiple cysts, the ovary may look macroscopically normal (Goldzeiher 1981). On cross section though, a characteristic appearance is revealed. Multiple small cysts (usually <10mm) are found aligned around the outer margin of the ovary.

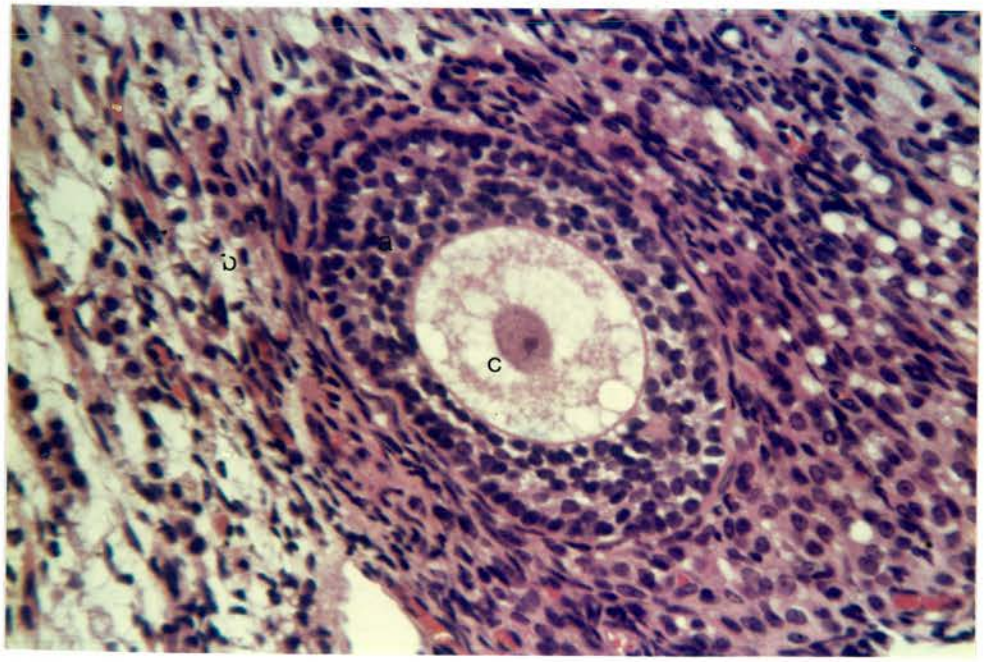
Histologically the capsule of the ovary, the tunica albuginea, is thickened and may be 15 times wider than normal (Goldzieher and Green 1962). Hyperplasia of the stromal theca cells has been described but is not a consistent feature (Green and Goldzieher 1965). In 1943, Fraenkel used the term 'hyperthecosis' in relation to polycystic ovaries. Although sometimes thought to constitute a separate disease, hyperthecosis more likely reflects a more severe form of the condition in which there is excessive hyperplasia of the luteinized theca cells surrounding the follicle (Futterweit 1984). These follicles have been found at varying stages of maturation and atresia. The follicular cyst walls are lined with granulosa cells which are separated from the theca cells by a basement membrane.

Biochemically the follicles were shown to be similar to preantral follicles, i.e. follicular fluid has high androstenedione and low oestradiol concentrations (Tanabe et al 1983). Also the intraovarian and the serum androstenedione and testosterone concentrations were elevated (Laatikainen et al 1980). In the absence of dominant follicle formation and hence a

Figure 1.7 POLYCYSTIC OVARY

GROSS APPEARANCE
a. Thickened capsule
b. Dense stroma

DEVELOPING FOLLICLE
a. Granulosa cells
b. Theca cells
c. Oocyte



corpus luteum, the ovarian production of oestradiol and progesterone remained low.

These ovarian features were originally thought to reflect a single disease entity. However, with the development of specific hormone assay techniques, it became apparent that several distinct endocrinopathies may be associated with polycystic ovaries (Yen 1980). These include Cushing's syndrome, congenital adrenal hyperplasia, hyperthyroidism, hypothyroidism and hyperprolactinaemia. Some early investigations of PCOS must have unwittingly included a variety of different diseases and it is not surprising that some of their results were therefore difficult to interpret. Current studies now exclude patients with these other specific problems and patients are now generally selected on the basis of the biochemical features consistent with our present understanding of the pathophysiology of the condition. This is explained in detail in the following section.

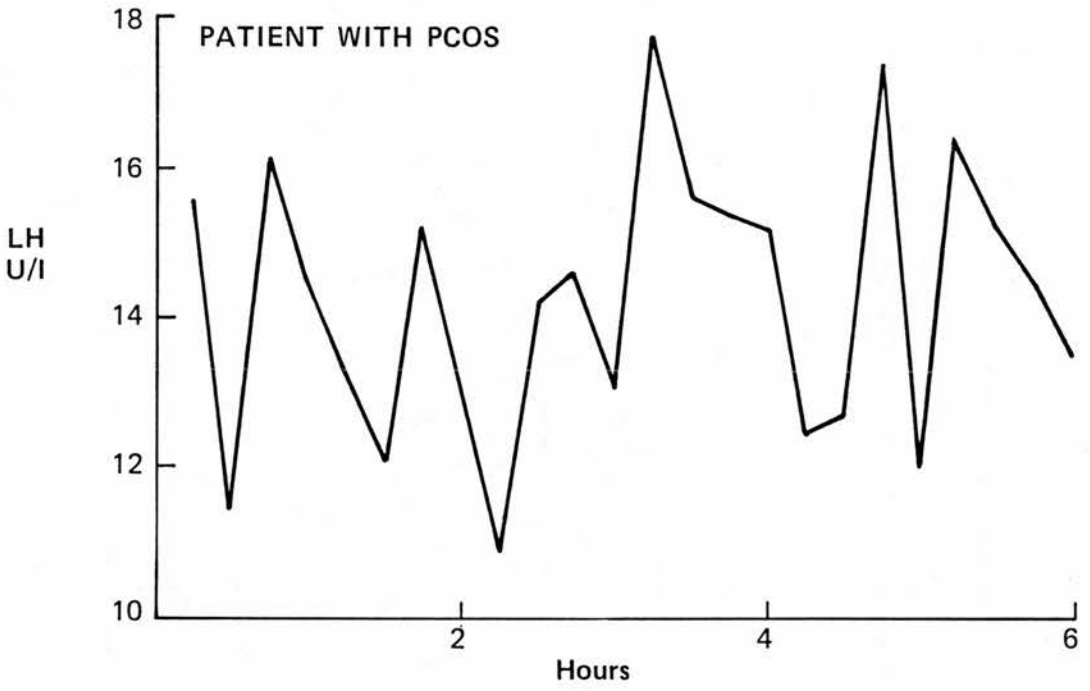
1.4.3 Pathophysiology of PCOS

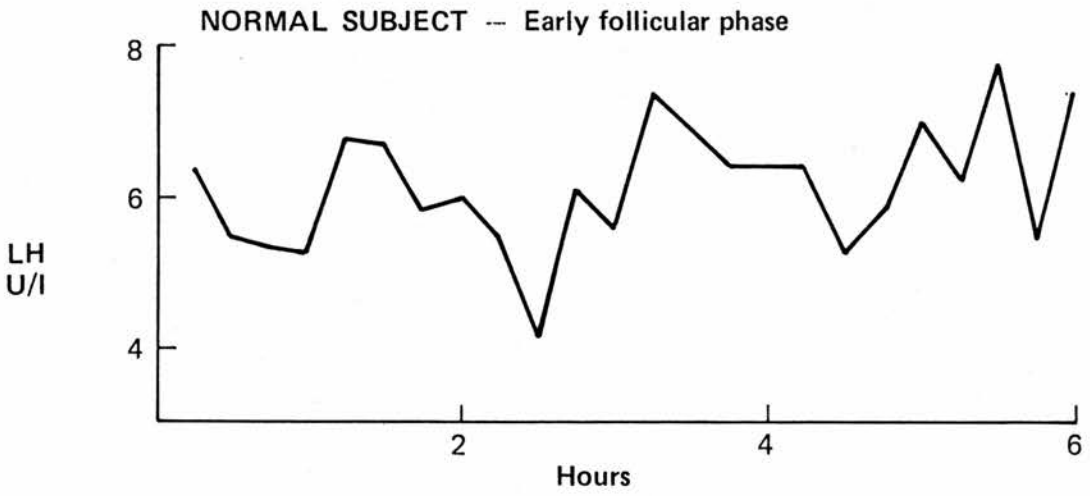
In his review of 1945 Stein stated his opinion that PCOS resulted not from a congenital, inflammatory or degenerative disease but from a definite endocrine disturbance. Although this has been confirmed by subsequent studies, the precise cause of the disturbance remains unknown.

The histological appearances of the ovary with hyperplasia of the theca cells suggests excessive stimulation of these elements. Consistent with this finding is the observation that the concentrations of the hormones produced by the theca cells, androstenedione and testosterone, are elevated in PCOS (de Vane et al 1975, Laatikainen et al 1980, Yen 1980). As described before, LH stimulates these theca cells and therefore LH has been studied in detail in PCOS. Not surprisingly, elevated concentrations of LH were found. Early workers using bioassays for gonadotrophins (McArthur et al 1958) revealed wide fluctuations in 'LH' activity. More sensitive radioimmunoassay techniques have enabled these patterns to be analysed in detail.

In PCOS concentrations of LH were found to be elevated whereas FSH concentrations were low or normal (Rebar et al 1973, Berger et al 1975, de Vane et al 1975, Baird et al 1977, Chang et al 1982). The pulsatile nature of LH secretion in PCOS has also been studied. The elevated mean concentrations were mainly due to increased amplitude of LH pulses (e.g. Figure 1.8) whilst no consistent frequency changes have been found (Rebar et al 1976, Baird et al 1977). In addition, the gonadotroph was thought to be hypersensitive to LHRH since exaggerated responses to exogenous LHRH were observed (Katz and Carr 1976).

FIGURE 1.8
EXAMPLES OF SERIAL LH DATA
Samples taken every 15 minutes for 6 hours





This abnormality is not thought to be due to an inherent pituitary defect since the normal negative and positive feedback responses to oestradiol remained intact (Shaw et al 1975a, Rebar et al 1976, Baird et al 1977). It is suggested that the increased pituitary sensitivity to LHRH may be due to chronic oestrogen stimulation. In normal women oestrogen administration enhanced the LH/FSH respones to LHRH (Jaffe and Keye 1974). Additional support for this theory was the strong positive corellation found between concentrations of the two oestrogens, oestrone and oestradiol, and LH concentrations in PCOS (de Vane et al 1975, Lobo et al 1981). Oestrogen concentrations were also related to the LH response to LHRH in PCOS (Rebar et al 1976).

LH secretion in PCOS can also be modulated in the normal manner by exogenous progesterone and this may explain the fall in LH concentrations which has been observed to follow an ovulatory cycle (Baird et al 1977, Rebar et al 1976).

If the LH elevation in PCOS is due to excess oestrogen stimulation, that implies that there must be abnormal oestrogen concentrations in this condition. Studies have shown that although oestradiol concentrations were normal, those of the weaker oestrogen, oestrone, were elevated (Baird et al 1977, Rebar et al 1976, de Vane et al 1975). The principal source of oestrone in women is from adipose tissue where enzymes were demonstrated which can convert androstenedione to oestrone (Siiteri and MacDonald 1973). As discussed already, androstenedione concentrations are elevated in PCOS. Since the ovary produces this hormone acyclically in PCOS, the resulting oestrone concentrations

are also chronically elevated. This is presumed to be the cause of the increased gonadotroph sensitivity.

The clinical symptom of hirsutism in PCOS is a result of the increased circulating androgens, in particular, androstenedione and testosterone. Hirsutism, by definition, refers to the rough quality of hair. Nonetheless, it is generally applied to women when the quantity of hair is increased. In its worst form, the normal male patterns of hair growth occur including a full beard. Hair follicles are sensitive to changes in skin androgen concentration although there is considerable variation between individuals. Androstenedione and testosterone are metabolised in the skin to the more potent androgen, dihydrotestosterone and this latter hormone stimulates the coarse terminal hair growth (Price 1975).

An additional factor which aggravates the problem is thought to be sex hormone binding globulin (SHBG) (Anderson 1974). When bound to SHBG, androgens are inactive. Thus, when SHBG concentrations are low, the relative androgenic activity at the target organ is increased. One factor which determines the concentration of SHBG is the proportion of oestrogens to androgens. Relatively high androgen concentrations cause a decrease in SHBG. Clearly with excess androgens in PCOS, it is not surprising that SHBG and therefore the active unbound androgen concentration is significantly raised (Hatch et al 1981, Yen 1980).

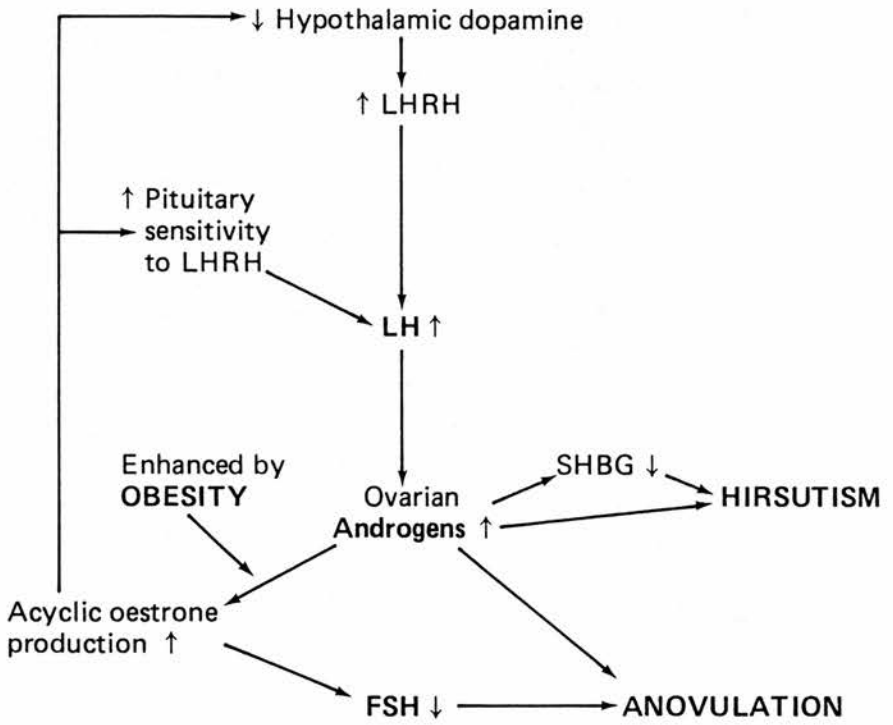
From the preceding evidence of the biochemical abnormalites found in PCOS, a hypothesis has been formulated which links these

observations and may be the explanation of the pathogenesis of the condition (Rebar et al 1976, Baird et al 1977). It is illustrated in Figure 1.9 and is summarised below.

Excessive LH stimulation of the theca cells results in increased ovarian androgen production. High intraovarian androgen concentrations block follicular growth and prevent ovulation. High plasma concentrations of androgens cause hirsutism and reduce SHBG concentrations thus aggravating the peripheral androgenic effect. Conversion of androstenedione in adipose tissue results in increased oestrone concentrations. This sensitises the gonadotroph to LHRH so that further excess LH secretion ensues. Being highly sensitive to oestrogen inhibition, FSH release is suppressed. In the absence of a rise in FSH to stimulate mature follicle growth, ovulation fails to occur.

More recently, a further factor has been implicated in the possible pathogenesis of PCOS. Evidence has accumulated which suggests that there is a hypothalamic deficiency of DA. This is based on three observations: firstly there is a suggested association between hyperprolactinaemia and PCOS, secondly DA infusion has been shown to cause an exaggerated fall in LH concentrations in PCOS, and thirdly a therapeutic role for the DA agonist bromocriptine has been claimed. This evidence is discussed in detail in Chapter 2.

FIGURE 1.9
SUGGESTED PATHOPHYSIOLOGY OF PCOS



CHAPTER 2: EVIDENCE FOR DOPAMINE DEFICIENCY IN POLYCYSTIC OVARY SYNDROME

2.1 PROLACTIN SECRETION IN PCOS

Since DA inhibits prolactin secretion, a deficiency would be expected to result in increased prolactin concentrations. Mild hyperprolactinaemia associated with PCOS has been reported in several studies although the incidence is variable; 67% (Corenblum and Taylor 1982), 44% (Buvat et al 1982), 22% (Carmina et al 1984). Variation in the definition of hyperprolactinaemia between studies may explain the differing results. As discussed earlier, many physiological events can elevate prolactin concentrations above the normal range e.g. stress, food and sleep. Therefore a single estimation of prolactin should not be used to diagnose hyperprolactinaemia. In practice it is usual to determine the basal prolactin concentration by taking several blood samples at short intervals in order to exclude temporary factors affecting the lactotroph. As stress may be induced by venepuncture, an indwelling intravenous cannula is often used for sampling.

Carmina et al studied 40 patients who had the typical symptoms of PCOS and were confirmed ultrasonically to have polycystic ovaries. They measured prolactin concentrations in 4 blood samples taken at half hourly intervals via an indwelling intravenous cannula. The mean of these samples was compared to those of 23 normal women in the early follicular stage of the cycle. Nine patients (20%) had mean prolactin concentrations greater than the normal range.

Buvat et al studied 16 patients with clinical symptoms of PCOS and laparoscopically confirmed polycystic ovaries. Three blood samples were withdrawn at 15 minutes from an indwelling

intravenous cannula at 8:00h, 12:00h and 16:00h. Each set was pooled and assayed for prolactin. Seven patients had at least one value greater than the normal range (44%) and four had two or more elevated values (25%).

Corenblum and Taylor investigated 25 women with clinical symptoms of PCOS. Blood samples were taken on three separate occasions and hyperprolactinaemia was diagnosed if the prolactin concentrations in at least 2 samples exceeded the normal range (16 control subjects).

Little consideration was given in any of these studies to the complex nature of spontaneous prolactin secretion which has been described earlier. This may explain the disagreement in results and also casts doubt on the existence of hyperprolactinaemia as a feature of PCOS. As hyperprolactinaemia has been a principal factor in favour of the DA deficiency theory, the evaluation of complex patterns of prolactin secretion in PCOS is clearly indicated.

The responses of the lactotroph cells to stimulation depends on the amount of prolactin stored in the pituitary and the availability of this prolactin for release. This may be a more sensitive indicator of changes in lactotroph control than is spontaneous secretion. Previous studies of lactotroph function in PCOS have given conflicting results. Prolactin responses to TRH in PCOS have been found to be either normal (Valkov and Dokumov 1977; Carmina et al 1984) or exaggerated (Falaschi et al 1980; Corenblum and Taylor 1982). In these cases the responses were found to be related to preceding prolactin concentrations. In a

study using the dopamine antagonist Haloperidol, an exaggerated reponse was seen which was confined to those patients who had elevated basal prolactin concentrations (Falaschi et al 1980).

A factor not taken into consideration in these studies which may explain the discrepancy in results, was recent ovarian function. The link between oestrogen, DA and prolactin has been discussed earlier and since oestrogen concentrations are high for two weeks preceding an ovulatory cycle, a difference might be expected between the lactotroph function of patients who have just ovulated and those who were amenorrhoeic. Lactotroph stimulation studies which consider this variable are needed.

2.2 EFFECTS OF DOPAMINE AND DOPAMINE AGONISTS IN PCOS

2.2.1 LH secretion

In a study of eight patients with PCOS, Quigley et al (1981a) demonstrated that a DA infusion promptly suppressed LH and the magnitude of the fall correlated with the basal LH concentration. The fall was significantly greater than that seen in normal women in the early follicular phase of the cycle. This increased sensitivity of the gonadotroph to DA was taken to be due to a reduction of endogenous DA inhibition of LH secretion.

Suppression of LH secretion has also been reported during treatment with the DA agonist bromocriptine. Following long term bromocriptine (5mg daily for 3 months), a reduction in LH secretion was observed in 13 of the 20 patients studied (Jaquet et al 1980). This response coincided with a restoration of ovulation in these subjects and it is therefore possible that the LH changes reflect the effects of other hormonal changes due to ovulation rather than a direct effect of bromocriptine. An attempt was made in their study to examine the pulsatile nature of LH release. However the long sampling interval and short total study period used meant that accurate analysis of the pulse patterns could not be made.

In a further study (Spruce et al 1984) 20 patients with PCOS whose main complaint was hirsutism, were treated with bromocriptine (7.5mg daily) for up to 1 year. A significant fall in LH concentrations was seen after 3 months therapy and this fall was maintained for 12 months. Again this coincided with a restoration

of menstruation which may account for the LH changes as described above. No attempt was made in their study to determine LH pulsatility. The LH response to LHRH was found to be reduced after treatment in both trials.

Pontirioli et al (1980) found that the acute administration of bromocriptine resulted in a fall in LH concentration. This has been taken as further evidence to support the hypothesis of decreased hypothalamic DA activity in PCOS. However, there are conflicting studies. Martin et al (1981) found that acute administration of bromocriptine did not alter LH secretion. Also when given as therapy to hyperprolactinaemic women, bromocriptine does not block physiological LH secretion since ovulation readily occurs (Thorner et al 1974, Strauch et al 1977, Klibanski et al 1984). Therefore, although LH secretion in normal women and patients with PCOS can be partially inhibited by DA infusion, the response to bromocriptine is variable.

One explanation for the confusion is the possible existence of several different DA receptors (Kebabian and Calne 1979). It may be that bromocriptine and DA act at separate receptor sites. DA stimulates D1 and D2 receptors whereas bromocriptine mainly stimulates D2 receptors. The sites of the various receptor types in man is unknown but animal studies suggest that the presynaptic receptors are D2 whereas the central postsynaptic receptors are the D1 type. Prolactin inhibition is mediated via D2 receptors and therefore is activated by both bromocriptine and DA. The postsynaptic DA inhibition of LHRH would involve D1 receptors only and hence the disparity in effects of bromocriptine and DAP. An alternative possible explanation for the inconsistent effects of

DA and DA agonists on LH concentrations is that in none of these studies were the detailed patterns of LH pulse secretion determined.

2.2.2 Therapeutic effects of dopamine agonists

On the assumption that DA may be deficient in PCOS it was logical to attempt to treat the condition with DA agonist therapy. Following reports of single cases of therapeutic benefit from bromocriptine, trials have been conducted which seem to confirm the initial observations (Jacquet et al 1980,Spruce et al 1984). Jaquet selected patients on the basis of the biochemical abnormalities consistent with the proposed pathophysiology of PCOS described earlier. Twenty patients were treated with 5 mg bromocriptine daily for three months. Thirteen had restoration of ovulation. The effect on hirsutism was not recorded.

In the study of Spruce et al 20 patients received 7.5 mg bromocriptine daily for between 3 and 12 months. Regular menstruation was restored in 12 of the 20 women and 11 of the 20 eventually noted an improvement in their hirsutism. This improvement was accompanied by decreases in androstenedione and testosterone although the absence of a change in SHBG makes the significance of the androgen change uncertain.

A major criticism of these trials is that they were not controlled and so a placebo effect cannot be excluded.

2.3 PROPOSED STUDIES

2.3.1 Studies of prolactin secretion in PCOS

The spontaneous secretion of prolactin in patients with PCOS will be compared with that of normal women taking into consideration the physiological variables such as the pulsatile pattern of secretion and the changes due to stress, food and sleep. In addition, the lactotroph reserve will be evaluated using various stimulating agents and the responses will be related to the preceding ovarian function.

2.3.2 Controlled trial of bromocriptine therapy

A double blind controlled trial will be reported to evaluate the therapeutic effects of the DA agonist, BRC, on patients with PCOS. The biochemical changes during therapy will be reported, in particular those of LH pulse patterns.

CHAPTER 3: STUDY METHODS

3.1 SELECTION OF PATIENTS

3.1.1 Diagnostic criteria

It will be clear from the preceding chapters that there is no single diagnostic criterion for PCOS. Patients who were selected for this study had biochemical features consistent with the proposed pathophysiology of PCOS.

The single clinical feature required was infrequent or absent ovulation dating from the menarche. Although obesity and hirsutism were common features, their presence was not considered to be essential for diagnosis. The biochemical criteria for diagnosis were an elevated LH concentration with raised LH to FSH ratio (>2:1) and androstenedione and testosterone concentrations at or above the upper limits of normal. Other specific endocrinopathies were excluded. Visualisation of the ovaries and histological diagnosis was not considered to be essential for diagnosis.

3.1.2 Patients and controls

The patients studied were recruited from general endocrinology or gynaecology clinics over a period of two years. Their presenting symptoms were oligomenorrhoea, amenorrhoea, infertility or hirsutism. The ages of the patients were 18- 32 years. The clinical and biochemical details of all the patients studied are given in Table 3.1. Normal women were recruited as control volunteers. They had regular menstruation and no hirsutism. Their ages were between 18-34 years. No medication was taken by any subject during the study. Local Ethical Committee approval was obtained and informed consent was given by all subjects.

TABLE 3.1: CLINICAL AND BIOCHEMICAL DETAILS OF PATIENTS STUDIED

*A = amenorrhoea
O = Oligomenorrhoea (6–12 weeks)
R = cycle <6 weeks

+Laparoscopy performed and polycystic ovaries found (no laparoscopy in other subjects)
×B = bromocriptine
P = placebo

													STUDIES PERFORMED (see text)							
SUBJECT	AGE	PREGNANCIES	* MENSES	WEIGHT kg	HEIGHT cm	HIRSUTE HIRSUTE	+ OVARIES	LH u/l	FSH u/l	LH: FSH	A nmol /l	T nmol /l	RANDOM PRL	FREQUENT SAMPLES	OVER-NIGHT PRL	DOMP	TRH	LHRH	1ST	× BRC TRIAL
1	22	0	A	82	167	+		8.7	4.0	2.2	19.3	4.6	+	+		+	+	+		+B
2	28	0	R	66	165	+	+	8.5	2.2	3.9	16.5	3.6	+	+		+	+	+		
3	31	0	R	73	167		+	13.2	5.3	2.5	9.8	3.2	+	+		+	+	+		
4	26	0	R	60	160	+		11.1	4.0	2.8	14.6	4.2	+			+	+	+		
5	21	0	0	47	160		+	13.5	3.4	4.0	10.8	3.9	+							
6	26	1	0	64	165	+		18.1	3.8	4.8	9.4	3.0	+	+		+		+		
7	31	2	0	47	155	+		22.0	3.6	6.1	18.2	3.6	+	+		+		+		+B
8	26	0	A	65	163	+	+	18.6	6.5	2.9	13.6	4.4	+							
9	32	2	A	85	165	+		19.7	2.7	7.3	18.9	3.3	+	+		+	+	+		
10	19	0	0	90	165	+	+	8.7	2.9	3.0	14.6	4.3	+							
11	22	0	0	68	163	+		12.0	3.0	4.0	17.9	4.3	+							
12	23	0	A	60	163	+		38.0	6.7	5.7	24.1	4.1	+	+		+		+		
13	20	0	A	60	155	+		17.8	6.6	2.7	9.3	3.5	+							
14	30	0	A	67	163	+	+	18.2	6.7	2.7	13.3	4.3	+	+		+		+		
15	18	0	A	99	163	+		10.5	2.9	3.6	13.0	5.5	+							
16	22	0	0	102	167	+		11.3	5.4	2.1	33.6	3.2	+	+		+		+		+B
17	18	0	0	56	158			19.3	6.5	3.0	12.3	2.8	+							
18	21	0	0	67	155	+		11.9	1.9	6.3	11.5	3.3	+	+			+	+		+B
19	23	1	R	56	161	+		7.6	2.4	3.2	4.3	3.0	+							
20	31	0	0	108	164	+		14.2	6.3	2.3	12.9	3.5	+							
21	22	0	0	59	164	+	+	32	5.2	6.2	11.4	4.2	+							
22	25	0	0	62	158	+	+	16.3	5.2	3.1	14.1	5.2	+							
23	29	0	R	54	164	+	+	13.4	4.5	3.0	12.7	4.3	+	+		+	+	+		+P

TABLE 3.1 (cont): CLINICAL AND BIOCHEMICAL DETAILS OF PATIENTS STUDIED

													STUDIES PERFORMED (see text)							
SUBJECT	AGE	PREGNANCIES	* MENSES	WEIGHT kg	HEIGHT cm	HIRSUTE HIRSUTE	+ OVARIES	LH u/l	FSH u/l	LH: FSH	A nmol /l	T nmol /l	RANDOM PRL	FREQUENT SAMPLES	OVER-NIGHT PRL	DOMP	TRH	LHRH	1ST	X BRC TRIAL
24	31	1	0	65	160	+	+	36.0	6.2	5.8	13.2	4.2	+							
25	28	0	0	51	158	+		16.2	6.3	2.6	16.1	3.8	+							
26	27	0	0	62	160	+	+	13.5	6.6	2.1	14.1	3.4	+	+		+		+	+	
27	18	0	A	91	164	+		10.9	5.4	2.0	12.8	3.2	+							
28	21	0	A	53	157	+		13.6	4.0	3.4	14.4	3.8	+	+		+		+		+P
29	32	3	R	90	155	+	+	19.2	3.3	5.8	11.2	4.4	+							
30	28	1	0	107	170	+		37.0	5.7	6.5	15.0	4.6	+							
31	32	1	0	120	160	+	+	16.1	6.6	2.4	14.0	6.7	+							
32	21	0	R	86	162	+		12.1	5.0	2.4	10.9	3.0	+							
33	28	0	0	106	170	+		17.5	5.5	3.2	9.8	4.3	+	+		+		+		+P
34	18	0	0	61	162	+		17.0	4.8	3.5	11.4	3.1	+		+					
35	26	0	0	81	160	+		32	5.2	6.2	10.0	3.5	+							
36	24	0	0	85	157	+		17.6	5.0	3.5	12.2	5.1	+					+		+P
37	32	2	0	51	153	+		11.4	3.7	3.1	42.0	4.0	+	+		+	+	+		+P
38	31	2	0	56	157	+		30.0	4.7	6.4	15.0	4.8	+							
39	21	0	0	55	15.6	+		29.0	5.6	5.2	11.3	3.4	+							+B
40	25	0	A	78	157	+		15.8	5.6	2.8	12.4	4.3	+	+		+		+		
41	22	0	0	71	155	+		27.0	4.8	5.6	11.4	3.6	+	+				+		+B
42	18	0	A	81	160	+		9.7	4.2	2.3	17.8	4.8	+	+						+B
43	18	0	A	52	162	+		6.6	2.4	2.8	14.9	3.5	+	+				+		+B
44	27	0	A	59	158	+		33	5.0	6.6	15.4	3.6	+							
45	18	0	0	54	160	+		11.1	2.9	3.8	11.7	2.5	+							
46	31	1	0	57	157	+	+	22.9	6.9	3.3	11.4	3.0	+					+		

TABLE 3.1 (cont): CLINICAL AND BIOCHEMICAL DETAILS OF PATIENTS STUDIED

													STUDIES PERFORMED (see text)							
SUBJECT	AGE	PREGNANCIES	* MENSES	WEIGHT kg	HEIGHT cm	HIRSUTE HIRSUTE	+ OVARIES	LH u/l	FSH u/l	LH: FSH	A nmol /l	T nmol /l	RANDOM PRL	FREQUENT SAMPLES	OVER-NIGHT PRL	DOMP	TRH	LHRH	1ST	X BRC TRIAL
47	29	1	R	76	156	+	+	13.1	5.6	2.3	19.5	4.2	+							
48	25	0	0	63	155	+	+	9.8	4.5	2.2	15.9	3.0	+	+				+		+P
49	24	1	0	92	164	+		15.3	4.4	3.5	18.8	6.8	+	+				+		+B
50	20	0	0	56	160	+		11.9	1.3	9.2	39.2	3.4	+	+				+		+B
51	25	0	A	73	161	+	+	11.2	3.5	3.2	9.5	4.5	+	+				+		+B
52	25	0	0	93	165	+		15.2	4.2	3.6	12.6	3.4	+	+				+		+P
53	24	0	0	58	155	+		18.0	4.5	4.0	27.1	4.0	+	+		+	+	+		
54	30	1	A	64	168	+	+	19.7	4.5	4.4	21.7	3.7	+	+				+	+	
55	29	0	A	60	170	+	+	29.0	6.5	4.5	14.7	4.6	+							
56	24	0	0	56	160	+		13.7	4.8	2.9	25.9	5.0	+							
57	33	2	A	72	160	+	+	8.9	3.9	2.3	12.1	3.1	+					+		+P
58	21	0	0	103	155	+		17.3	4.0	4.3	12.6	3.7	+	+		+	+	+		+P
59	19	0	0	57	152	+		30.7	5.3	5.8	13.0	4.2	+							
60	22	0	0	51	154	+		11.0	4.6	2.4	14.2	4.2	+							
61	23	1	0	64	155	+	+	9.6	2.4	4.0	12.1	3.9	+	+						
62	27	1	A	70	165			22.0	4.7	4.7	12.5	3.5	+							
63	21	0	0	64	160	+		8.4	2.3	3.7	12.6	3.2	+							+P
64	26	0	0	67.5	157	+	+	16.5	3.2	5.2	17.0	4.3			+		+		+	
65	31	0	0	93	165	+	+	14.5	6.7	2.2	14.5	4.2			+		+		+	
66	24	0	0	65	160	+		12.2	4.1	3.0	12.2	3.4			+					
67	30	0	0	72	163	+	+	27.0	5.1	5.3	18.6	3.3							+	
68	32	0	0	64	152	+	+	23.0	7.0	3.3	13.8	1.4							+	
69	25	0	0	62	165		+	24.0	6.1	3.9	15.3	4.3							+	

3.2 HIRSUTISM ASSESSMENT

The rate of hair growth was measured photographically by a modified method of Burgess and Edwards (1978). The area to be measured (24x36mm) was shaved and a control photograph taken. A second photograph was taken 5 days later. No hair growth could be measured on the initial photograph, therefore the measured growth in the second photograph was recorded as representing five days of growth.

A glass slide was placed firmly over the skin to flatten the hairs. A Nikon FM2 camera with a 105 mm macro lens and extension ring was used. With the lens at maximum extension the primary magnification was x 1 (i.e. life-size image). A small electronic flash was mounted on an angular bracket at the front of the lens and once the optimum flash position was established this was retained throughout the trial. The camera was hand held and with the lens at maximum extension the object distance was altered until a sharp image was achieved. The negative was enlarged and printed to a magnification of x3.85 (Figure 3.2) on an automatic printer. The image distance was noted enabling precise standardisation of subsequent prints.

From each print the number of growing hairs was counted. In addition the total length of 10 hairs on each print was recorded using dividers as described by Burgess and Edwards and hence the growth could be calculated.

The error due to the depth of field of the lens was determined by photographing and printing a 10 mm image 10 times. The coefficient of variation of the method was 0.5%. The error in

Figure 3.2 EXAMPLE OF 5 DAYS OF HAIR GROWTH.

measurement of growth rate was determined by 10 repeat measurements of 1 print and was 5% at 0.41 mm/day. All measurements were made blindly and in random order by the same observer.

3.3 ASSAY METHODS

All samples were separated within 12 hours of the test and were stored at -20°C until assay. Samples were assayed in duplicate and mean values used. For the frequent sampling study, all samples from one subject for LH and prolactin were assayed together. In the dynamic tests of lactotroph function, all samples from each test were assayed together.

Radioimmunoassay techniques were used to measure the hormones in this study. This method depends upon the competitive binding of an antibody with a standard quantity of radiolabelled hormone and unlabelled hormone (the antigens). The latter is the unknown concentration within the test sample. Excess antigen is present in order to enhance the competition for binding sites. The unbound antigen is separated from the immune complexes by the addition of a second precipitating antibody. The radioactivity in the precipitate is related to the amount of displaced labelled antigen and hence the concentration of hormone in the test sample. A standard curve is constructed by plotting logit B/Bo against log concentration of the standard where B=amount of radiolabelled antigen bound in the presence of standard amounts of unlabelled antigen, and Bo= amount of radioactively labelled antigen bound in the absence of unlabelled antigen. From the linear part of this plot the unknowns can be read. Intra- and interassay coefficients of variation can be determined by assay of pooled sera containing concentrations of antigen covering the range of standards used.

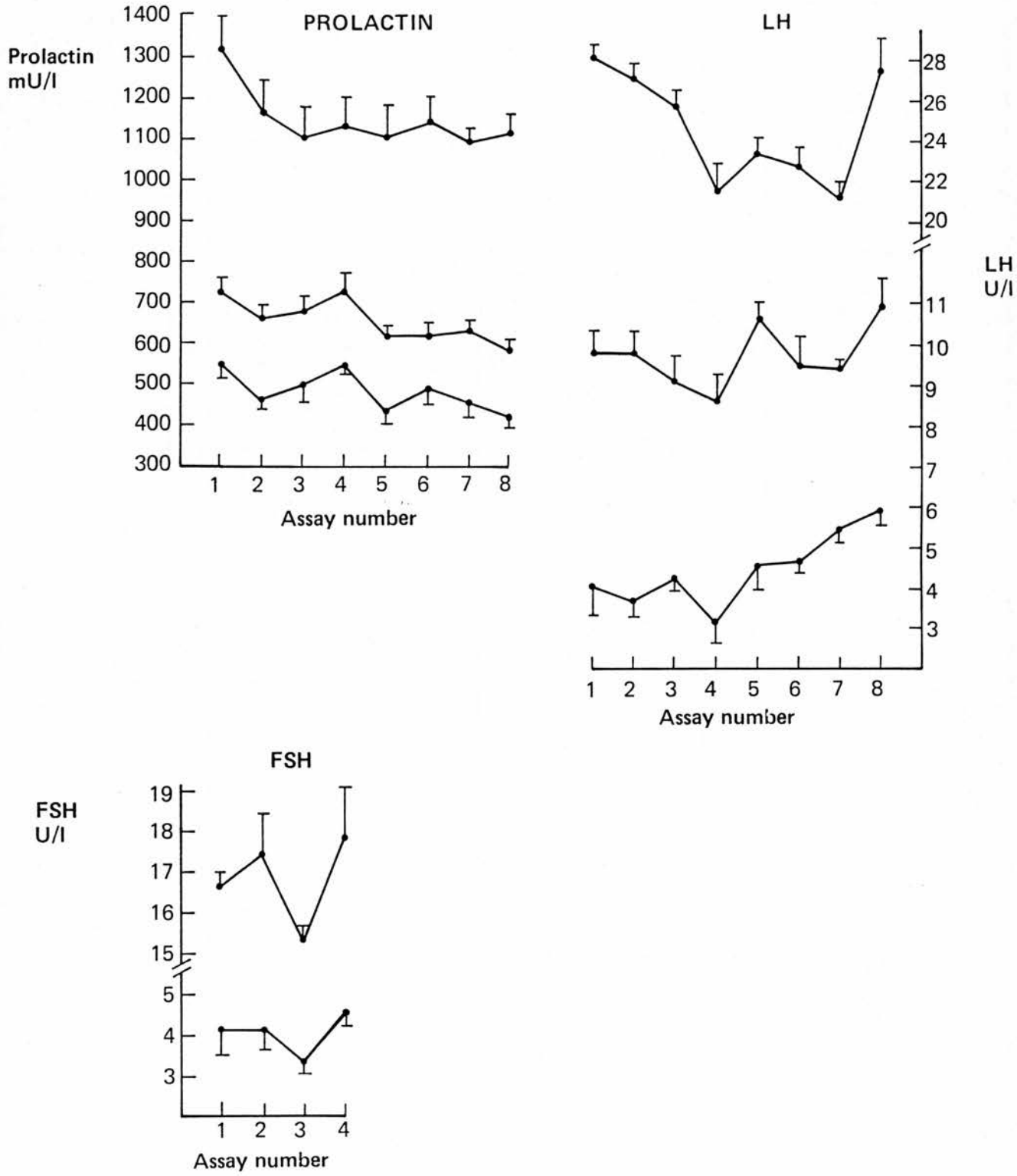
3.3.1 LH, FSH and prolactin assay

LH and FSH were assayed using standards WHO 68/40 for LH and WHO 78/549 for FSH. The LH and FSH antibodies were obtained from Dr K. Ferguson, Chelsea Hospital. Prolactin was assayed using standard NIBSC 81/541 and the antibody was obtained from RIA UK. The intra assay coefficients of variation were <7.5% and <4.9% at 9.7 U/l and 25 U/l LH: and <12.1% and<7.0% at 4.0U/l and 16.8U/l FSH. The interassay coefficients of variation were <10.8% and <11.4% for LH and <15% and <7% for FSH. The intra-assay coefficient of variation for prolactin were <9.6% and <7.4% at 379 mU/l and 1037 mU/l. The interassay coefficient of variation was <13.0%. These variations are illustrated in Figure 3.3.

FIGURE 3.3

INTER- AND INTRA-ASSAY VARIATION

Prolactin, LH and FSH concentrations of 3 different pooled human sera were estimated 10 times in each assay. The mean (+ SD) of the pool in each assay are shown.



3.3.2 Steroid assays

Routine laboratory radioimmunoassay techniques were used for steroid assays.

Oestrone assay involved ether extraction prior to radioimmunoassay which used antibody obtained from Steranti Research Ltd (Product Code E001) and radiolabel [2,4,6,7-3H]-oestrone (Amersham International plc). The interassay coefficients of variation were <8.8% and <5.9% at 352pmol/l and 764pmol/l.

Oestradiol was assayed using an I^{125} oestradiol direct radioimmunoassay kit supplied by Steranti Research Ltd (Product Code ER155). The interassay coefficient of variation was <6.6% and <3.0% at 334 pmol/l and 963 pmol/l.

Testosterone was measured by radioimmunoassay following extraction with ether, using rabbit antibody (Steranti Reseach Ltd Product Code A003) and [1,2.6,7-3H]-testosterone (Amersham TRK402) as label. The interassay coefficients of variation were <9.4% and <8.6% at 1.8 nmol/l and 10.4nmol/l.

Androstenedione analysis involved ether extraction then radioimmunoassay using antibody obtained from RIA Ltd (Product No. 1025) and [1,2,6,7-3H]-androstenedione as label. The interassay coefficients of variation were <7.4% and <5.4% at 3.4 nmol/l and 5.6 nmol/l.

The technique for measurement of SHBG was a saturation assay using dihydrotesosterone as ligand. The interassay coefficient of variation was <9%.

CHAPTER 4: STATISTICS

4.1 STUDENT'S t-TEST

The two sample t-test was used to compare mean incremental changes of observations of two groups after treatment. The significance of the difference between the means was determined by estimating the t value (mean of the differences divided by the standard error of the mean) and reading off the probability value at n-1 degrees of freedom from the table of t distribution.

For comparison of the mean observations between two different groups, the non-paired t-test was used. The t value was calculated as the difference between the means divided by the standard error of the difference between the means. The probability value was read off the table of t distribution at n-1 degrees of freedom.

4.2 NON-PARAMETRIC TESTS

When the data for analysis did not have a normal distribution, non-parametric tests were used. Wilcoxon's rank sum test (two sample test) was used to compare 2 groups of data. All observations were ranked in order and the rank total for each group was added separately. The probability value for difference between the groups was read from the appropriate statistical table.

4.3 PULSE ANALYSIS

The difficulties of interpreting serial biological data have been recognised (Yates 1981, Clifton and Steiner 1983, Merrian and Wachter 1982). Two main problems were appreciated. Firstly, there was imprecision in the methods of radioimmunoassay. Random variations due to assay error may have been interpreted as physiological pulses (false positive error) or may have masked genuine pulses (false negative error). This problem was particularly important when the original pulse amplitude was small relative to the random assay error. The second problem was that of the sampling interval and the total study period which were necessarily limited for practical reasons. Using the method of analysis employed in this study the shortest frequency detectable was 60 minutes (sampling interval x4) and the longest was 6 hours (total study period). The number of cycles in the study period and the number of samples taken per cycle also affected the analysis (Clifton and Steiner 1983, Veldhuis et al 1984).

Several different approaches have been made to the problem. The most commonly employed analyses used threshold methods (Yen et al 1972, Santen and Bardin 1973, Baird et al 1977, Merriam and Wachter 1982) i.e. peaks were identified from features such as height and shape. The 'frequency' was then determined by counting the number of peaks occurring in the study period. These methods were limited by the problems mentioned above. Whilst being adequate methods for analysing sporadic events and correlating events such as coincident LH and FSH pulses, their value for

detecting cycles was restricted. A cycle detector programme (Clifton and Steiner 1983) has been devised based on a variable threshold method which offered some solution to the problem of equating false positive and negative errors but was limited by the requirement of relatively homogeneous amplitude pulses. Alternative methods which depend on assumptions about the distribution of data have been employed (Christian et al 1978). The presence of peak skewed the distribution of the observed values. The analysis depended on the separation of the peak values from the normally distributed baseline. This method proved to be insensitive when compared to threshold methods (Merriam and Wachter 1982). Conventional methods of time series analysis have been employed in studies in the cow (Rahe et al 1980) but it has generally been assumed that since they were not applicable to the analysis of irregular cycles, their value to the analysis of LH data was limited. It had previously been assumed that LH secretion was irregular although there was no basis for this assumption.

For this study three methods of pulse analysis were applied. The first two represented the methods most commonly employed at the time:

1. For the analysis of prolactin series the more sensitive method of Santen and Bardin was used. A pulse was defined as a rise over two successive samples of greater than twice the coefficient of variation of the assay. The amplitude of the pulse was the difference between samples.

2. For the analysis of serial LH data, Baird et al (1977) considered a pulse to have occurred if two consecutive samples (peak samples) were greater than the previous two samples (basal samples) and the value of one exceeds the 95% confidence limits of the mean of the two basal samples. The magnitude of the pulse was measured by subtracting the mean of the basal samples from the highest peak value.

3. The third method employed, time series analysis (in particular spectral analysis), was chosen because it was felt that detection of an underlying regular harmonic pattern in the data may have been a better estimate of the physiological signal than the threshold methods of pulse detection.

A spectral analysis of a time series is a decomposition of the total variation in the series into harmonic components represented by cyclic variations at frequencies corresponding to 1,2,3...n/2 complete cycles in time T (n = number of observations, T = total study time). The highest frequency detectable corresponds to a series of alternating high and low values; whilst the lowest frequency includes any long-term trends extending beyond the total period of observation. Estimates of these harmonic components of variation are plotted as a periodogram e.g Fig 6.4. Each ordinate represents the amount of variation in the series attributable to a harmonic frequency represented by the abscissa value. The usual null hypothesis is one of 'white noise'. Informally this means that there are no important harmonic components of variation, so that all periodogram ordinates should

be equal, within the limits of statistical fluctuation. A more formal definition of white noise is that the series consists of the observed values of independent, identically distributed random variables. A test of significance is available, the 'cumulative periodogram test'. Less formally, 95% 1-sided tolerance limits can be added to the periodogram to indicate which, if any, harmonic components are important. Under white noise, each periodogram ordinate lies above these limits with probability <0.05. The periodogram ordinates are mutually independent. The mathematical details of these methods are given in Priestley (1981); a more elementary but incomplete account is in Chatfield (1980, Chapter 7).

CHAPTER 5: STUDIES OF PROLACTIN SECRETION IN PCOS

5.1 SPONTANEOUS PATTERNS OF PROLACTIN SECRETION IN PCOS

5.1.1 Design of the study

Sixty-two patients with PCOS and 28 control subjects were investigated. The clinical and biochemical details of these patients are given in Table 3.1. All studies on menstruating women, except the random samples, were performed in the early follicular phase of the menstrual cycle (Day 2-7). In most of the patients with oligomenorrhoea, ovulation in the preceding cycle was determined by weekly blood sampling for estimation of plasma progesterone until the onset of menstruation. Progesterone concentrations greater than 20 nmol/l were considered to indicate ovulation. All patients classified as anovulatory had progesterone concentrations less than 2 nmol/l or had amenorrhoea for at least 3 months before and 1 month after the study.

Random serum prolactin concentrations were estimated from 62 patients and 20 control women. Subjects in whom this was greater than the upper limit of the normal reference range in our laboratory (>450 mU/l) were studied further. An indwelling intravenous cannula was inserted and prolactin estimations were made then and again after 1 and 2 hours.

Twenty-eight patients with PCOS and 10 normal women had the details of their spontaneous prolactin secretion assessed by a frequent sampling study. They were admitted to the Investigation Unit at 08:00h. An intravenous cannula was inserted into the forearm and from 08:30h samples were withdrawn every 15 minutes for 6 hours. Subjects rested supine during the study. Lunch was taken at 12:00h.

Four patients and 6 controls had hourly blood samples obtained from an indwelling intravenous cannula overnight during sleep. They were admitted at 18:00h after a meal and the cannula was inserted at 19:00h. Subjects rested during the evening and retired to bed at about 20:30h after a hot drink. All were observed to be asleep during most of the night.

5.1.2 Results

Two of the 62 patients had persistent, mild elevation of prolactin concentration when cannulated samples were taken (>450<1500mU/l). The multiple sampling study was performed on one of these patients (Table 8.1, subject 42). Her mean prolactin concentration was elevated over each two hour study period compared with that of the PCOS group ($t = 11.65, 7.35, 6.27$, $p<0.001$). The clinical features and other biochemical measurements of these individuals were not unique.

The mean random prolactin concentrations of the remaining patients are shown in Table 5.1. When several samples had been taken from one subject, the mean was considered. The distribution of results was skewed to the lower concentrations so non-parametric tests were used for comparison. There was no significant difference between the patients and the controls. At least one random prolactin estimation was greater than the laboratory normal range (450mU/l) in 19 of 62 patients and six of 28 controls (differences not significant).

More accurate basal prolactin estimations were obtained from the multiple sampling studies (Table 8.1). Fluctuations occurred in all series suggestive of pulsatile secretion. Pulses were defined by the more sensitive method of Santen and Bardin (1973) rather than the method of Baird et al (1977) which was specifically applied to the interpretation of LH series. There was no obvious regular pattern in these pulses. The mean number of fluctuations per patient was 3 per 6 hours (range 1-6) and for the

TABLE 5.1
RANDOM PROLACTIN CONCENTRATIONS

	PCOS	Controls
No. of subjects	60	28
Mean mU/l	313	319
Range mU/l (all samples)	100–945	118–881 Differences not significant

Actual values (mean if several random samples from one subject)

PCOS						Controls		
105	309	315	305	387	435	355	193	310
313	483	290	185	190	323	118	550	241
218	380	516	352	195	670	371	881	191
342	285	155	390	170	195	219	239	221
267	170	385	216	350	467	214	272	282
237	205	490	244	436	255	201	577	272
110	342	308	135	230	503	212	224	223
362	485	201	205	225	245	548	238	320
377	556	410	445	183	325	476	475	
451	303	318	277	177	322	196	323	

controls was 2 per 6 hours (range 1-4). These are illustrated in Figure 5.1.

Also illustrated are two variant patterns detected frequently in the series; initial prolactin elevations and increments after lunch. For comparison of the changes in prolactin concentrations within individual series, an initial elevation was considered to have occurred if the mean of the first 2 hours of the study exceeded the 95% confidence limit of the mean of the middle two hours of study. Similar criteria were used to define a post-prandial rise in prolactin concentration by comparing the means of the middle and last two hours of the study.

(1) Initial prolactin elevations are demonstrated in Figure 5.1. Such changes were seen in 21 of 28 patients and 9 of 10 controls (difference not significant). The prolactin fall was completed by 2 hours after cannulation in all subjects.

(2) Prolactin increments after lunch were seen in 14 of 28 patients and 3 of 10 controls (difference not significant). Because of these observations, the mean prolactin concentrations were compared after dividing each 6 hour study period into 3 x 2 hour series. The results are shown in Table 5.2. There was no difference between the patients and the controls in any of these groups.

No difference was found related to recent ovulation.

The four patients and six controls who were studied overnight had similar sleep related prolactin rises (Figure 5.2 and Table 8.2).

FIGURE 5.1

EXAMPLES OF THE INDIVIDUAL SPONTANEOUS PATTERN OF PROLACTIN SECRETION OVER 6 HOURS TO SHOW:
(a) SPONTANEOUS FLUCTUATIONS
(b) INITIAL ELEVATION (c) POST PRANDIAL ELEVATION

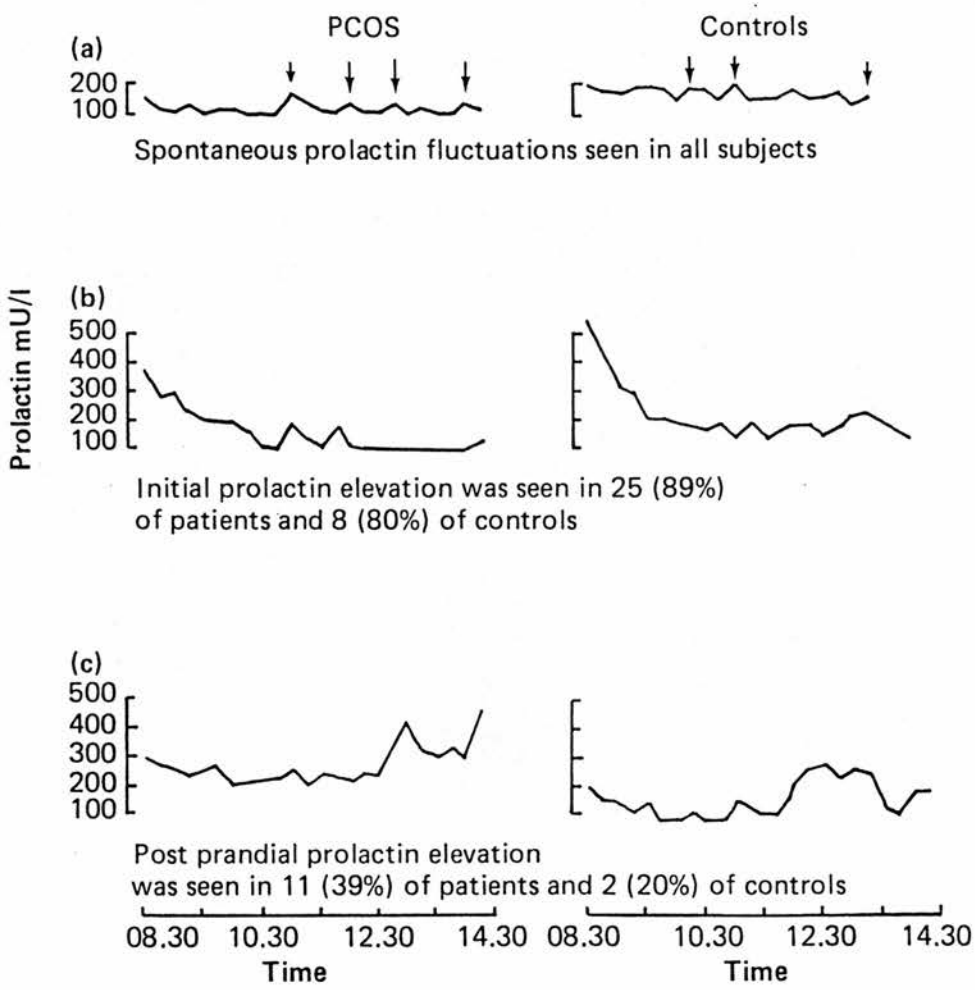
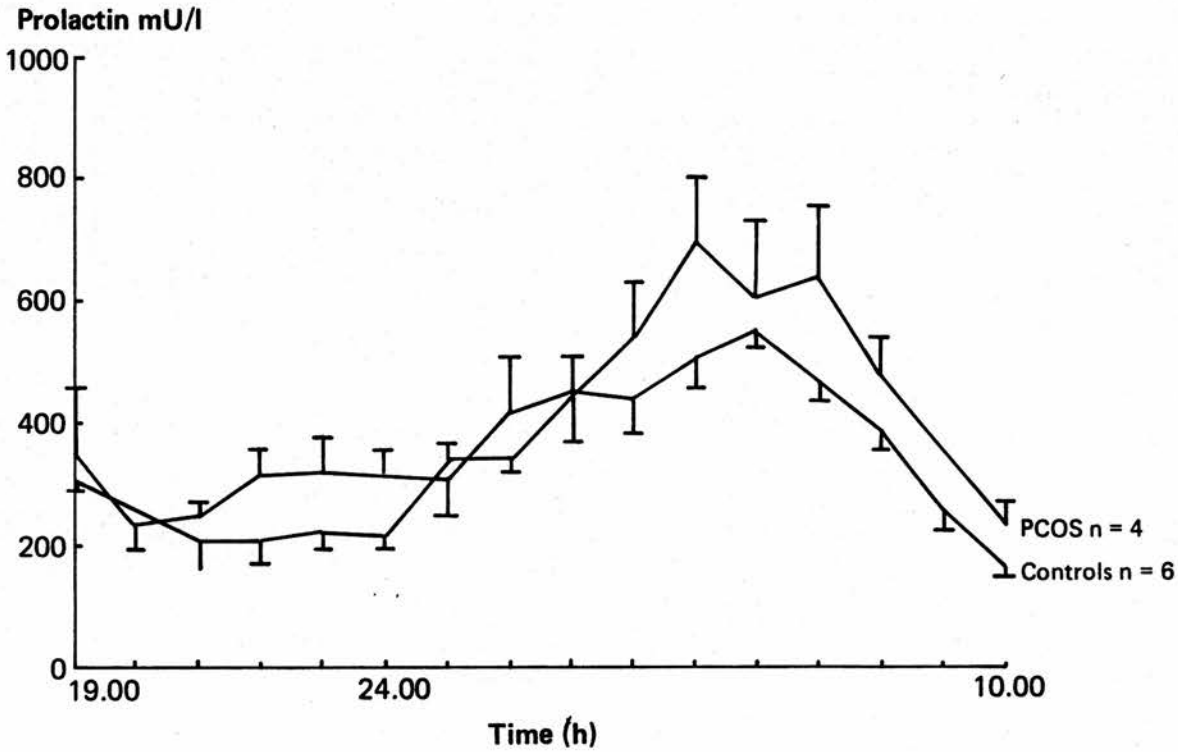


TABLE 5.2
MEAN PROLACTIN CONCENTRATIONS TAKING ACCOUNT OF THE INITIAL ELEVATION (0–2 h), POSTPRANDIAL ELEVATION (4–6 h) AND THE RECENT OVARIAN FUNCTION

	PCOS			Controls
	All	Ovulatory	Anovulatory	
No. of subjects	28	6†	15†	10
Mean (range) mU/l				
0–2 h	228 (108–417)	199 (108–280)	243 (143–417)	220 (105–333)
2–4 h	177 (100–424)	151 (100–205)	180 (104–424)	157 (100–226)
4–6 h	204 (103–494)	201 (146–275)	214 (103–494)	178 (101–374)
			Differences not significant	

†Ovulation not determined in all subjects

FIGURE 5.2
OVERNIGHT PROLACTIN CONCENTRATIONS (mean ± SE)



5.1.3 Discussion

In this study, the incidence of mild hyperprolactinaemia was two in 62 patients. In the remaining subjects, no abnormality of prolactin secretion was found. These two subjects may have the unusual condition of 'hyperprolactinaemic PCOS' described by Yen (1980). He considers this to be a syndrome of unknown and probably separate aetiology from PCOS. Therefore the patients were excluded from the study group because the aim of the investigation was to examine the lactotroph function as it related to the aetiology of PCOS. However if these patients simply reflect a more severe form of the disease their results should be included. The data were reanalysed accordingly but no significant difference was found.

The discrepancy between the results of this study and the earlier findings (Corenblum and Taylor 1982, Buvat et al 1982, Carmina et al 1984) may be explained by the problems of both patient selection and the diagnosis of hyperprolactinaemia. Because of the nature of their symptoms, patients with PCOS do not always seek medical attention. Hence neither this study nor the others can represent the true incidence of hyperprolactinaemia. The patients in this study were referred for further investigation from general endocrinology and gynaecology clinics over a period of two years but it is unlikely that all individuals with PCOS were seen. Some may have been referred to a separate research clinic for the investigation of hyperprolactinaemia. To examine this possibility, the records of 102 women aged between 16 and 40

years seen for hyperprolactinaemia over the same period were reviewed. Only two patients were found who fulfilled the criteria for diagnosis of PCOS used in this study. Both had the additional symptoms of galactorrhoea and neither had hirsutism. They responded to bromocriptine with regulation of menstruation. Inclusion of these subjects in this series increased the incidence of hyperprolactinaemia in PCOS to 7%.

In addition there was a potential inconsistency in the diagnosis of PCOS which determined patient selection. As described earlier (Section 2.1), Corenblum and Taylor relied on clinical features alone and the studies of Carmina et al and Buvat et al used clinical and ovarian observations for diagnostic purposes rather than the biochemical findings. Despite the differences in diagnostic criteria, their mean basal steroid and gonadotrophin concentrations were consistent with those in this study. It seems probable therefore that we were dealing with the same pathological condition.

Variation in the definition of hyperprolactinaemia between studies may explain the differing results. As discussed earlier, many physiological events can elevate prolactin concentrations above the normal range e.g. stress and food and sleep. Therefore a single estimation of prolactin should not be used to diagnose hyperprolactinaemia. In this study the diagnosis was made only if all three estimations were above the normal reference range. Carmina et al used the mean of a similar series and application of their method to the current data did not alter the incidence of hyperprolactinaemia. Applying the definition of Buvat et al (mean

of three samples taken at 15 minute intervals) the incidence of hyperprolactinaemia in this study rose to 5%, and using the definition of Corenblum and Taylor (2 of 3 random samples greater than the normal range) the incidence became 13%. In the latter study, all but two of the 12 hyperprolactinaemic patients had at least one prolactin estimation within the normal range. Therefore by the definition used here persistent hyperprolactinaemia occurred in only two of their 18 patients.

Intermittent prolactin elevations were noted in both the patients and controls. As similar increases were present in both normal women and patients, they cannot reflect a specific abnormality of PCOS. The spontaneous fluctuations of prolactin may reflect a regular pulsatile pattern of secretion similar to that found for LH (see Chapter 6) but such a pattern could not be determined under the experimental conditions used here. However, an abnormality of pulsatility seemed unlikely since the mean prolactin concentration was not affected. Furthermore, the physiological significance of such an abnormality in relation to ovarian regulation is uncertain since the pattern of prolactin secretion does not appear to affect ovulation (Klibanski et al 1984).

5.2 LACTOTROPH RESPONSE TO STIMULATION IN PCOS

5.2.1 Design of the study

The clinical and biochemical details of the patients in these studies are given in Table 3.1. Ovulation in the preceding cyle was determined as in Section 5.1.1.

(1) DOMPERIDONE TEST

The reponse of the lactotroph to DA antagonists was tested using domperidone (Janssen Pharmaceuticals) which blocks DA receptors (Pourmand et al 1980). This drug does not cross the blood brain barrier so effects the median eminence of the hypothalamus without having widespread actions in the central nervous system. It is therefore devoid of the side effects commonly associated with other DA receptor blocking agents such as metoclopramide which can enter the central nervous system. Since the lactotroph response to domperidone and metoclopramide are similar (Pourmand et al 1980, Judd et al 1976), the former drug was selected. Seventeen patients with PCOS and 13 control women were studied. After an overnight fast, an intravenous cannula was inserted into the forearm at 08:30h and blood was taken then and after 15 minutes for basal estimation of prolactin. Domperidone (10mg) was given intravenously and further samples of blood were taken after 15, 30, 45, 60, 90 and 120 minutes for prolactin estimation. No side effects were noted.

(2) THYROID RELEASING HORMONE (TRH) TEST

This test has been described in Jacobs et al (1971). Intravenous administration of the synthetic preparation of TRH (Roche) to normal subjects induces a prompt rise in prolactin; the magnitude of which reflects the amount of prolactin stored and available for release in the lactotroph. Eleven patients with PCOS and 8 control women were studied. An intravenous cannula was inserted into the forearm at 14:00h and basal serum samples were obtained then and after 15 minutes. Thyroid releasing hormone (200ug) was given intravenously and blood was obtained after 20 and 60 minutes for prolactin estimation. The typical side effects of flushing and a metallic taste were noted by some individuals.

(3) LUTEINISING HORMONE RELEASING HORMONE (LHRH) TEST

The ability of LHRH to effect a release of prolactin was discussed in Section 1.2. A synthetic analogue (Ayerst Pharaceuticals) was used in this study. The response of the lactotroph to LHRH was tested immediately after the multiple sampling study described in Section 5.1.1. After the last of the multiple samples was taken (14:30h) LHRH 100 ug was given intravenously and further blood samples were taken after 20, 40 and 60 minutes for prolactin estimation.

(4) INSULIN STRESS TEST

The mechanism by which stress induces a rise in prolactin is uncertain but is probably mediated via the hypothalamus. In order to determine the lactotroph response to stress, insulin

is given to produce hypoglycaemia. The presence of prolactin release following stress is physiological and the magnitude of the response represents the quantity of prolactin available for release in the lactotroph.

Hypoglycaemia was induced with insulin in eight patients with PCOS and seven normal women. They were admitted to the Investigation Unit at 08:00h after an overnight fast. All had a recent normal electrocardiograph. Soluble insulin 0.1U/Kg body weight was given to lower the blood glucose concentration to less than 2nmol/l. At this point all had the typical symptoms of sweating and lightheadedness. Blood samples from an indwelling intravenous cannula were obtained 15 minutes before and at the time of insulin injection and again after 30, 60, 90 and 120 minutes.

At the time of these studies, a blood sample was also taken for estimation of oestrone, oestradiol, androstenedione, testosterone, sex hormone binding globulin (SHBG), LH and FSH. When more than one test was performed on an individual they were separated by at least 24 hours.

5.2.2 Results

The results of studies using the lactotroph stimulating agents domperidone, TRH and LHRH are given in Table 8.3 and are illustrated in Figures 5.3, 5.4 and 5.5. The prolactin responses to domperidone and TRH were clearly related to the preceding ovarian function. After an ovulatory cycle, a normal response was seen whereas a blunted response followed a period of anovulation.

Responses to LHRH were comparatively small and there was no difference between the groups.

FIGURE 5.3
PROLACTIN RESPONSES TO DOMPERIDONE
(100 mg IV) (mean ± SE)

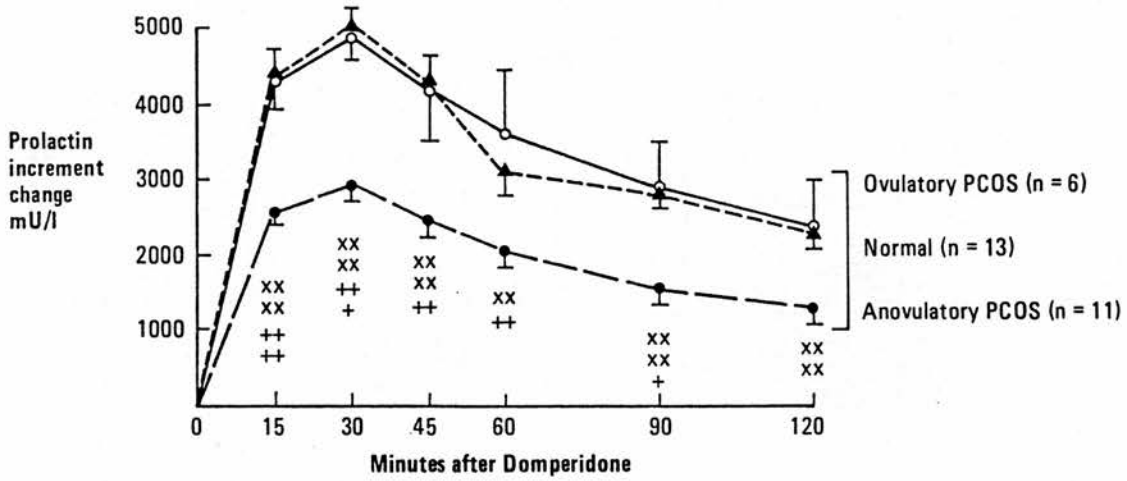


FIGURE 5.4
PROLACTIN RESPONSES TO TRH (200 μg IV)
(mean ± SE)

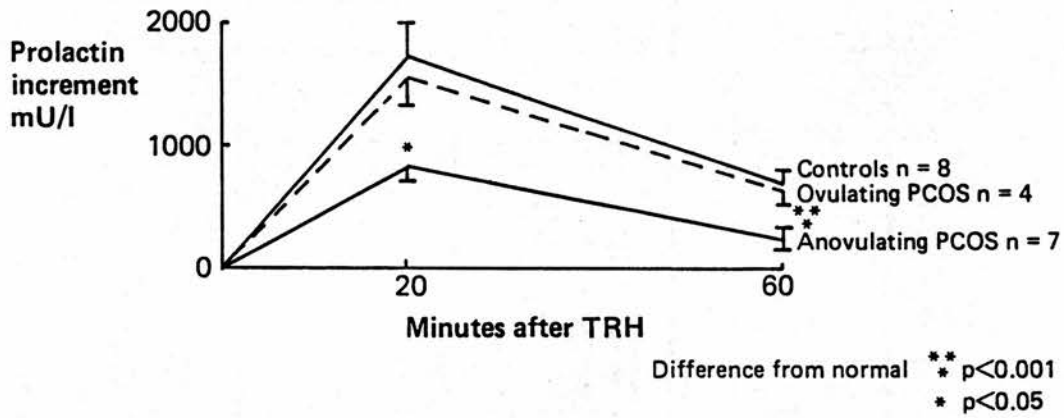
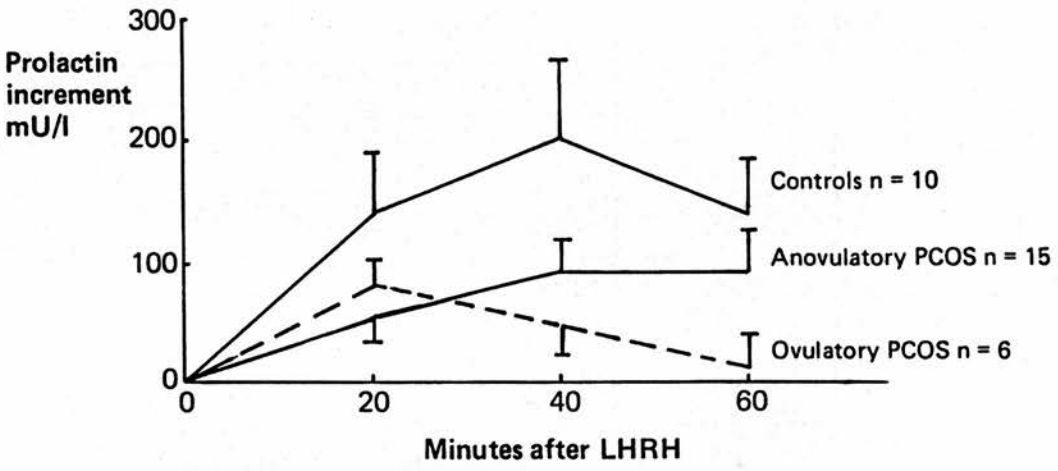
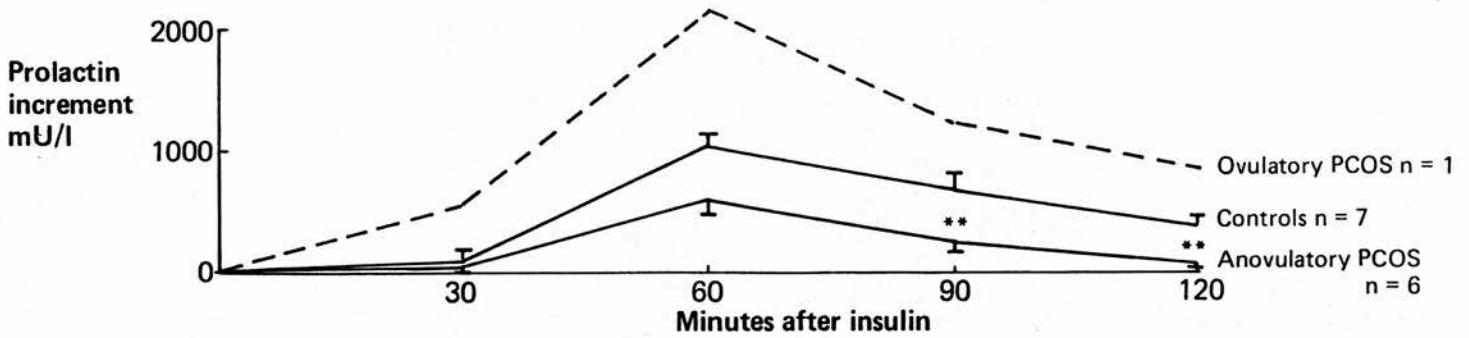


FIGURE 5.5
PROLACTIN RESPONSE TO LHRH (100 μg IV)
(mean ± SE)



Insulin induced hypoglycaemia caused a prolactin increase and again this was found to relate to recent ovarian function. The prolactin response in PCOS was blunted in the 6 patients who were amenorrhoeic at the time of the study (Figure 5.6). A seventh patient who had ovulated, had an apparently exaggerated response and her basal prolactin concentration was high (400 mU/l). Basal prolactin concentrations were not related to the maximum per cent increase in any of the other dynamic tests in these studies.

FIGURE 5.6
PROLACTIN RESPONSE TO INSULIN INDUCED HYPOGLYCAEMIA
(mean ± SE)



Difference from normal ** $p < 0.01$

Basal concentrations of testosterone, androstenendione, oestrone, oestradiol, SHBG, LH and FSH determined at the time of the study are shown in Table 5.3. As expected, high concentrations of testosterone, androstenedione, oestrone and LH were found whereas oestradiol and FSH concentrations were normal. SHBG was low but the difference overall was not significant. Similar biochemical changes were seen in all patients but the significant differences from normal were confined to the anovulatory group.

TABLE 5.3

BASAL STEROID AND GONADOTROPHIN CONCENTRATIONS AT THE TIME OF STUDY (mean ± SE)

	PCOS			Controls
	All	Ovulatory	Anovulatory	
No. of subjects	28	6‡	15‡	10
Testosterone (nmol/l)	3.9 ± 0.2**	3.7 ± 0.6	4.1 ± 0.3**	2.8 ± 0.2
Androstenedione (nmol/l)	17.3 ± 1.5**	21.1 ± 5.3	17.8 ± 1.5**	11.5 ± 1.2
Oestrone (pmol/l)	368 ± 25**	330 ± 146	425 ± 25**	227 ± 18
Oestradiol (pmol/l)	184 ± 20	138 ± 13	210 ± 32	152 ± 20
Sex hormone binding globulin (nmol/l)	37 ± 21	43 ± 7	27 ± 1.8**	63 ± 11
LH (U/l)	12.7 ± 1.2**	8.4 ± 0.9**	16.3 ± 1.8**	4.9 ± 0.3
FSH (U/l)	4.3 ± 0.3	4.1 ± 0.4	4.6 ± 0.2	3.6 ± 0.4

Differences from normal ** $p<0.001$

** $p<0.01$

‡Ovulation not determined in all subjects

5.2.3 Discussion

Unlike earlier reports, these studies have failed to show an abnormality of lactotroph reserve specifically related to PCOS. However, none of the earlier studies took into account the preceding ovarian cycle in the patients. It was clearly shown here that recent ovulation determined the lactotroph response to stimulation. Other studies of patients with various anovulatory conditions such as hypothalamic hypogonadotrophic amenorrhoea (Quigley et al,1980), normogonadotrophic secondary amenorrhoea (D'Agata et al,1981), and normogonadotrophic and hypogonadotrophic primary amenorrhoea (Batrinos et al, 1981) have revealed similar blunted prolactin responses to dopamine blockade. It seems probable therefore that the blunted prolactin responses seen in PCOS reflect anovulation rather then any specific lactotroph abnormality in PCOS.

The reasons why ovulation may alter lactotroph function are unknown. Oestrogens are probably involved as has been discussed earlier. In women who had ovulated there was a high concentration of oestradiol in the two weeks preceding the study which may have stimulated the lactotroph. The absence of this in anovulatory women may be the reason for the blunted lactotroph to stimulation.

High concentrations of progesterone are also seen in the luteal phase and its possible role in the alteration of lactotroph response to stimulation has yet to be determined.

An alternative explanation for the different results of the ovulatory group is that they may have constituted a separate or less severe disease group. To some extent this was confirmed by

the basal steroid and gonadotrophin concentrations (Table 5.3). The effect of ovulation on the biochemistry in PCOS has already been discussed and it is clear that improvement may be observed immediately after an ovulatory cycle. By waiting for the early follicular phase in oligomenorrheic patients, it was therefore not surprising to find less biochemical aberration in these subjects. When the biochemical parameters used for the initial diagnosis were analysed, no difference was found between the ovulatory and anovulatory patients.

Obesity is a common feature of PCOS. In this study the mean weight of the patients was 26% $\pm$ 33% above the average (Life Extension Institute of New York City). Studies of prolactin secretion in massively obese women (103-104% above Ideal Body Weight) have shown either normal responses to TRH and the DA antagonist, sulphiride, (Sannia and Benna 1982) or blunted responses to TRH and absent response to insulin stress testing (Kopelman et al 1980). In the latter study the abnormal reactions remained after weight reduction suggesting an underlying hypothalamic disorder of prolactin regulation in some women with previous obesity, irrespective of their weight at the time of the study. This could be an explanation for the results of the study reported here. However, no correlation was found between the patient's weight and the lactotroph response.

The hypothesis being tested in this study has made the assumption that the origin of the hypothalamic DA which may regulate LHRH secretion is similar to that which inhibits

prolactin secretion. Figure 1.3 illustrates the different ways by which DA may modulate LHRH secretion. In man the precise neurons involved with LHRH regulation in the median eminence have not been distinguished from those which release DA into the portal vessels and hence inhibit prolactin release. It is possible therefore that a DA deficiency effecting LHRH secretion may not alter the DA regulation of the lactotroph. This might explain the lack of alteration of lactotroph function in PCOS observed in this study.

CHAPTER 6: STUDIES OF LONG-TERM DOPAMINE AGONISTS IN PCOS

6.1 DESIGN OF THE STUDY

A previous study (Spruce et al 1984) of the therapeutic effects of the DA agonist bromocriptine in PCOS found that 75% of the patients had improved menstrual frequency and 55% had improvement in their hirsutism. Anticipating that a similar response would be seen in a controlled trial, it was calculated from the statistical tables of Casagrande et al (1978) that with 22 patients receiving either bromocriptine or placebo, there would be an acceptable probability ($p<0.05$) of a significant result. Patients were allocated randomly to either bromocriptine or placebo. The placebo was an inert compound of similar appearance to the bromocriptine tablet and was provided by Sandoz UK Ltd. Allocation of the patients to each group was unknown to patient or observer. Twenty-two patients were recruited over a period of one year. All had the main complaint of hirsutism. To confirm this objectively the scoring system of Ferriman and Gallwey (1961) [adapted by Hatch et al (1981)] was used. In this study, nine areas at which hair growth is hormonally dependent were graded 0-4, according to the amount of hair present. These areas were upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, upper arm and upper leg. Since only 5% of premenopausal women have total scores of greater than 8, this was taken to be the criterion for diagnosis of hirsutism. The clinical and biochemical details of the patients are given in Table 3.1.

In all patients the drug was given in slowly increasing dose from 1.25 mg to 7.5 mg daily over two weeks. Patients were

assessed at the start of the trial and again after 6 and 12 months of therapy.

Eleven patients received bromocriptine; 7 completed 12 months therapy, 1 defaulted for social reasons and 3 because of side effects. Eleven patients received a placebo; 9 completed 12 months, 1 defaulted for social reasons and 1 because of side effects. The side effects were those recognised to occur with bromocriptine i.e. nausea and dizziness.

6.2 HIRSUTISM STUDY

6.2.1 Methods

Hirsutism was assessed subjectively as 'worse', 'unchanged' or 'improved' (sufficient to warrant a request for continuation of treatment after the trial). Objective assessment of the response was by the photographic methods described in Section 3.2. Two areas were measured on each subject; the sideburn or chin and the midline subumbilical area. These were the sites which caused greatest concern. The precise areas were recorded relative to fixed anatomical landmarks such as the ear or umbilicus so that the measurements could be accurately repeated.

6.2.2 Results

Results of the subjective assesment of hirsutism are shown in Table 6.1. Two patients on bromocriptine reported a marked improvement in hirsutism but despite this observation both still needed to shave daily. The results of the photographic measurements are shown in Table 8.4 and 8.5, and are represented graphically in Figures 6.1 and 6.2. There was no significant difference in the change of hair growth rate or numbers of hairs between the groups. The individuals who reported an improvement in hirsutism are identified and no change was seen in the photographic assessments of these patients. These patients are discussed further in the next section.

TABLE 6.1
SUBJECTIVE ASSESSMENT OF HIRSUTISM CHANGE

		Bromocriptine n = 11	Placebo n = 11
Completed trial		7	9
Hirsutism:	Improved	2	–
	Unchanged	5	6
	Worse	–	3

FIGURE 6.1
HAIR GROWTH RATE DURING TRIAL

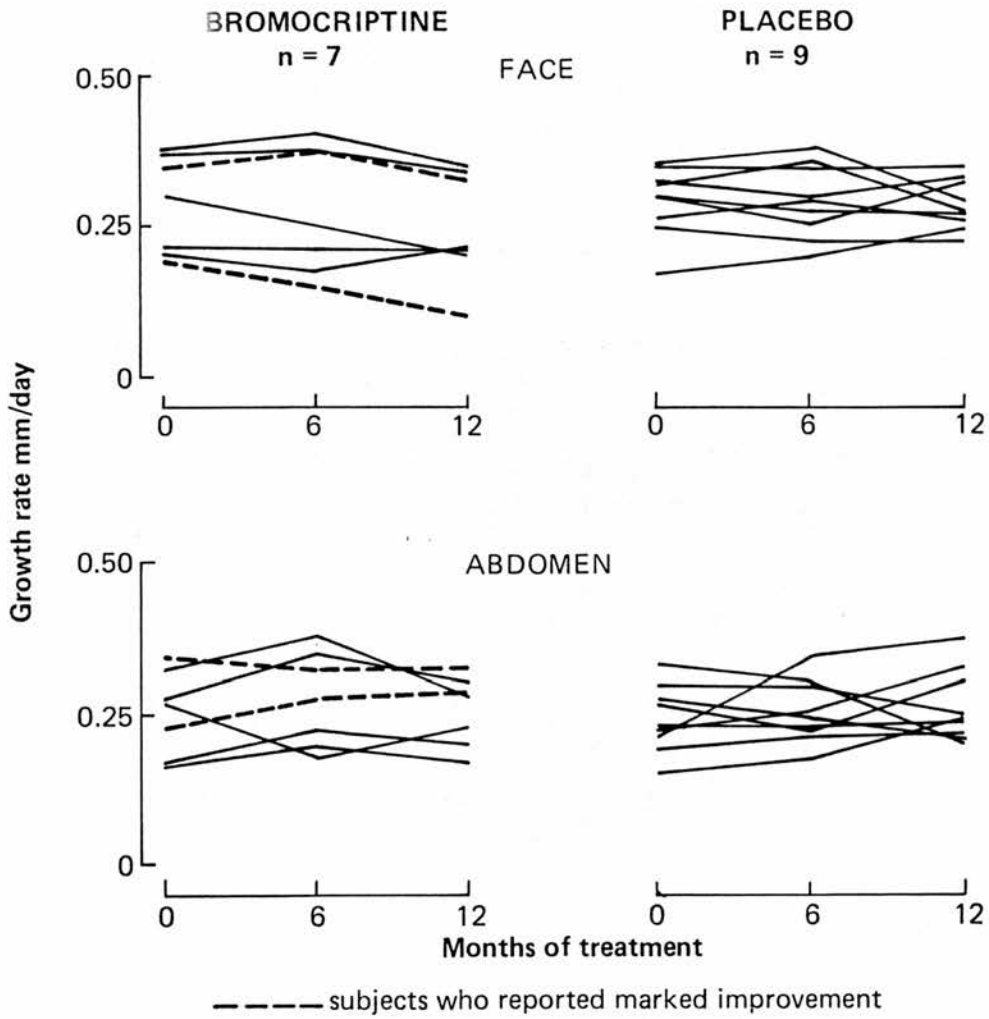
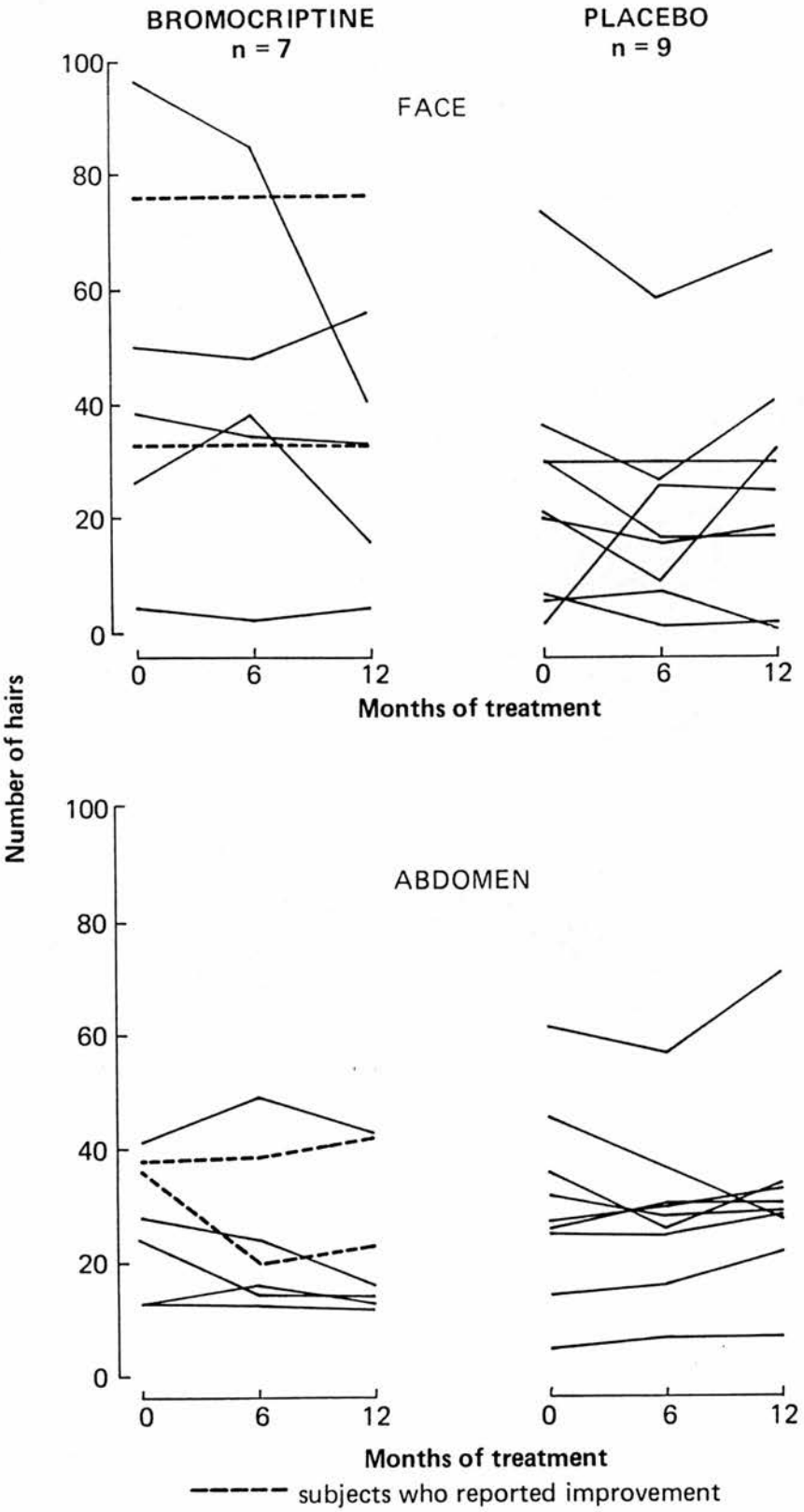


FIGURE 6.2
NUMBER OF HAIRS GROWING DURING TRIAL
(area 24 x 36 mm)



6.3 MENSTRUAL FREQUENCY STUDY

6.3.1 Methods

The change in frequency of menstruation between the 6 months preceding the trial to the last 6 months of therapy was compared between the two groups.

6.3.2 Results

There was no statistically significant difference between the alteration in menstrual frequency of the groups (Table 6.2). One of 2 subjects who reported improvement in hirsutism had more frequent menstruation.

The clinical and biochemical features of these two patients at the time of selection for the trial were not unusual (subjects 16 and 42 in Table 3.1). However, subject 42 was found subsequently to have persistent mild hyperprolactinaemia and was discussed in more detail in Section 5.1.3.

TABLE 6.2
MENSTRUAL PATTERN CHANGES
Number of periods per individual in 6 months

BROMOCRIPTINE (n = 7)			PLACEBO (n = 9)		
Subject No.	6 months pretrial	Last 6 months trial	Subject No.	6 months pretrial	Last 6 months trial
42	4	6	28	0	0
39	3	4	33	2	5
16	5	3	36	4	0
1	1	5	23	5	5
41	2	2	37	4	5
49	1	5	48	5	6
46	5	6	58	3	1
			52	2	3
			57	1	0

6.4 GONADOTROPHIN STUDY

6.4.1 Methods

In order to evaluate any change in gonadotroph function which may follow DA agonist therapy, the frequent sampling study outlined below was performed on most subjects.

Patients were admitted to the Investigation Unit at 08:00h. An intravenous cannula was inserted into the forearm and from 08:30h samples of blood were withdrawn every 15 minutes for 6 hours. Subjects rested supine during the study. After the final blood sample was taken a bolus of 100ug LHRH was given intravenously. Further samples were taken after 20, 40 and 60 minutes to determine the gonadotroph response to stimulation. In oligomenorrheic women, these studies were performed in the early follicular phase of the ovarian cycle and ovulation in the preceding cycle was determined as described in Chapter 5.

6.4.2 Results

The multiple sampling study was performed on 14 subjects altogether; 7 taking placebo were studied on 3 occasions and 1 was studied twice before defaulting because of side effects. Six subjects on bromocriptinewere studied on 3 occasions and 4 had pretrial studies only (all the latter defaulted on therapy). The results are shown in Table 8.6 and summarised in Table 6.3.

There was no statistically significant difference in the change in mean LH, FSH or LH:FSH ratio between the bromocriptine and placebo groups. The maximum response of LH and FSH to LHRH was also unaffected by treatment.

TABLE 6.3
GONADOTROPHIN CHANGES DURING TRIAL (mean values)

	BROMOCRIPTINE (n = 7)			PLACEBO (n = 9)		
	0	6 months	12 months	0	6 months	12 months
LH u/l	11.8	10.5	11.5	9.4	9.2	8.9
FSH u/l	4.0	3.4	3.7	4.2	4.2	4.0
LH : FSH	3.0	3.0	3.1	2.3	2.5	2.2
Max LH to LHRH	40	36	52	28	32	27
Max FSH to LHRH	3.2	2.7	2.3	2.6	3.7	3.2
Number of pulses LH	2.8	3.8	3.2	3.6	3.3	3.4
Pulse amplitude	2.6	3.8	3.3	2.5	3.3	2.3

Differences not significant

No change was found in LH pulse amplitude or frequency in either group when analysed by the methods of Baird et al (1977). Spectral analysis of individual series failed to reveal significant periodogram peaks in most cases.

6.5 STEROID STUDY

6.5.1 Methods

At the start of the trial and after 6 and 12 months a single blood sample was taken for estimation of the following; androstenedione, testosterone, oestradiol, oestrone and SHBG.

6.5.2 Results

The biochemical changes in each group are shown in Table 8.7 and summarised in Table 6.4. A significant fall in androstenedione was seen in each group but there was no difference between the changes in the bromocriptine and placebo groups. There was a difference in testosterone concentrations between the groups at the initial assessment. This difference was not seen in the pre-trial values (Table 3.1) and is therefore of uncertain importance.

TABLE 6.4
STEROID CHANGES DURING TRIAL (mean values)

	BROMOCRIPTINE (n = 7)			PLACEBO (n = 9)		
	0	6 months	12 months	0	6 months	12 months
Testosterone nmol/l	4.3	3.2	3.7	3.2†	2.9	3.1
Androstenedione nmol/l	21.0	10.2	12.4	15.7	11.3	9.0
Oestrone pmol/l	342	294	329	388	287	342
Oestradiol pmol/l	183	151	227	141	155	173
SHBG nmol/l	28	36	34	40	49	44

Comparison of basal concentrations between groups (non-paired t test)

† $p < 0.05$ No significant differences in incremental changes between groups

6.6 DISCUSSION

This trial has failed to find a therapeutic role for bromocriptine in PCOS. The biochemical improvements previously observed with this treatment were not confirmed.

6.6.1 Comparison with other studies

The studies of Jaquet et al(1980) and Spruce et al (1984) suggested encouraging therapeutic results from the use bromocriptine in the treatment of PCOS. This was associated with an improvement in the biochemical parameters.

Hirsutism was assessed in the trial of Spruce et al who used subjective methods to evaluate the changes in hair growth. Although improvement was reported by 50% of the patients, this was only 'marked' in two (10%). The difference between the results of the two trials was therefore not great. The failure to objectively confirm changes in hair growth may have been due to the methods used.

Hirsutism can be assessed relative to three parameters of hair growth; the rate of hair growth, the number of growing hairs and the thickness and colour of individual hairs. Photographic techniques as described in this study measured the first two parameters only. The thickness of hair can be measured by cutting them and using a eyepiece micrometer as described by Ebling et al 1977). Attempts to apply this method proved unsuccessful because there was a great variation in thickness of individual hairs within one subject (Figure 3.2). It was not feasible to measure

all the hairs present nor was it possible to randomly select hairs for evaluation. There is no method available for assessing hair colour changes.

In the trial reported here, menstrual frequency increased in both groups of patients suggesting that this may have been a placebo effect, although the precise mechanism by which this could have occurred is unknown. A placebo response may therefore be the reason for the significant improvement in menstrual frequency noted in the earlier studies.

All patients who had an increased frequency of menstruation were pleased, emphasising the pyschological benefit of regular menstruation to many women. In such patients the use of clomiphene to induce regular ovulation or oestrogen/progesterone preparations to induce regular menstruation may be preferable especially since some of the latter have been shown to cause a slight improvement in hirsutism (Dewis et al 1985).

The fall in LH concentrations and LH:FSH ratio, and reduction of the gonadotroph sensitivity to LHRH noted by Spruce et al may be explained by the improvement in menstrual frequency that was recorded (12 of the 20 patients had a regular menstrual cycle whilst on treatment). A similar improvement in gonadotrophin secretion was reported by Jaquet et al in the 13 of their 20 patients who had a restoration of regular menstruation. As discussed earlier, a decrease in LH concentration and reduction of LH:FSH ratio has been observed to follow an ovulatory cycle (Baird et al 1977). Although ovarian function was not determined by

Spruce etal, Jaquet et al had presumptive evidence of ovulation from biphasic basal body temperature charts. Thus gonadotrophin changes previously attributed to bromocriptine therapy may be a direct result of restoration of ovulation. Likewise the improvements in androstenedione and testosterone (Jaquet et al) and in testosterone (Spruce et al) which were not confirmed in the present study, may also be a result of the increased frequency of ovulation.

6.6.2 Trial design problems

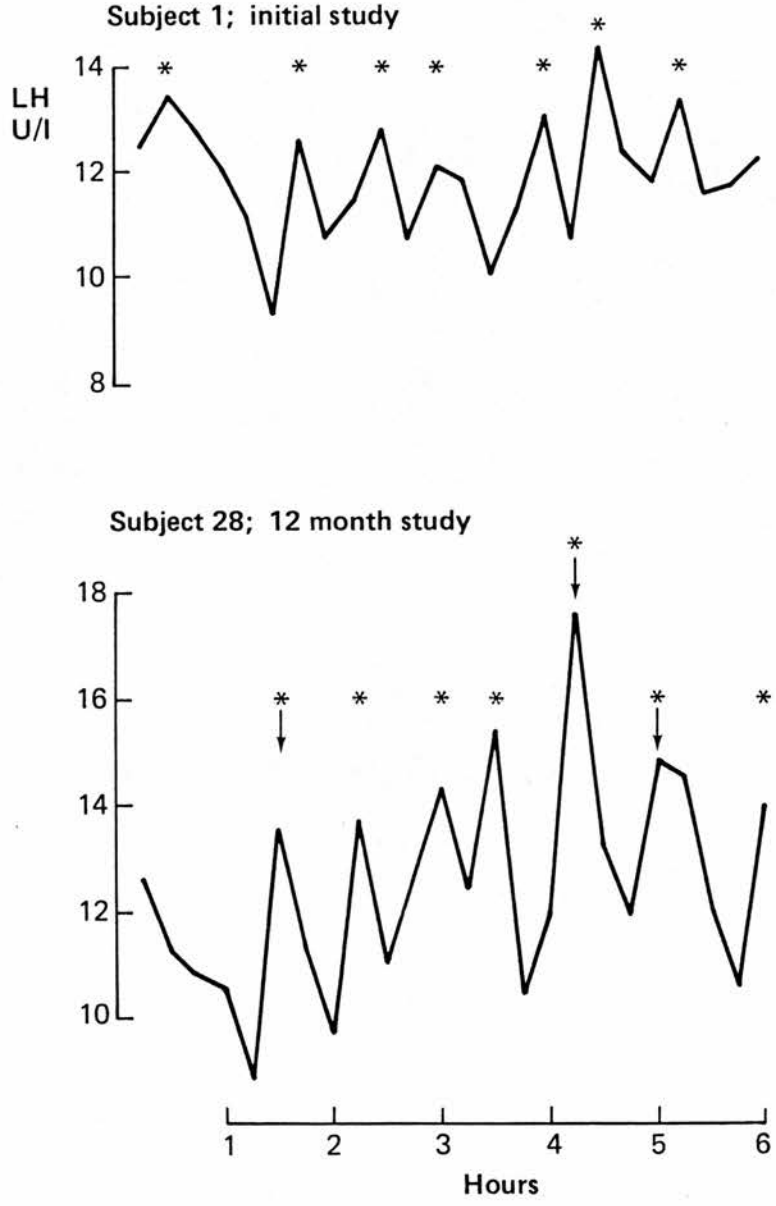
Bromocriptine is a drug with specific well recognised side effects. Since an inert placebo was used, the comparison of therapeutic responses could therefore be challenged. It was not considered ethical to administer a placebo with similar side effects. However, in practice not all patients on therapy experienced side effects and one patient taking placebo withdrew because of the typical symptoms of nausea and dizziness usually associated with bromocriptine.

An error was made in choosing the sample size for this study as no allowance was made for defaulters from therapy. The preliminary trial (Spruce et al 1984) reported results having excluded defaulters and therefore overestimated the responders. Only 13 of their original 20 patients completed 12 months therapy (a 35% default rate). Taking this into account, the sample size for a controlled trial should have been larger. Because of this, the study reported here has insufficient numbers to provide conclusive results. Despite this it is relevant to note that the two patients who reported improvement on therapy were still markedly hirsute. Therefore, it is unlikely that a major therapeutic role for bromocriptine exists.

6.6.3 Problems of pulse analysis

Figure 6.3 shows two sets of LH data analysed by the methods of Baird et al (1977) and Santen and Bardin (1973). The importance of the method of analysis is clearly illustrated. Three pulses are detected by the former analysis and 14 by the latter. This shows how much the results depend on the statistics used.

FIGURE 6.3
EXAMPLES OF LH SERIAL DATA
(15 minute sampling interval)



↓ Pulse defined by Baird *et al* 1977
* Pulse defined by Santen and Bardin 1973

The failure of time series analysis to detect regular pulses may have been due to the long sampling interval used. In order to evaluate this, LH data from control studies on 11 normal women were analysed. This work is partly described in Murdoch et al (1985). The protocol was similar to that described earlier as applied to the patients with PCOS except that the sampling interval was decreased to 5 minutes (6 subjects) or 10 minutes (5 subjects). The LH data obtained are given in Table 8.8. The results gave clear confirmation of the existence of regular harmonic patterns of LH secretion. The null hypothesis ('white noise') was emphatically rejected for all 11 subjects using the cumulative periodogram test ($p<0.001$). Three patterns emerged:

(1) LOW FREQUENCY VARIATION

Strong peaks at the lowest frequency (6h) were seen in 6 of the 11 subjects (e.g. Figure 6.4 and 6.5). These were indicative of a slowly varying change in LH concentrations during the period of observation.

(2) HIGHER FREQUENCY VARIATION

Clear periodiogram peaks were seen in 10 of the 11 subjects (e.g. Figure 6.4 and 6.5). The cycle lengths which correspond with these peaks are given in Table 6.5.

(3) RAPID OSCILLATIONS

One subject exhibited a strong peak corresponding to a cycle length of 72 minutes and in addition showed equally strong peaks at two higher frequencies corresponding to cycles of 20 and 13.1 minutes (e.g. Figure 6.5). Peaks at the same

frequency were also seen in eight other subjects although they were less strong (e.g. Figure 6.4).

Spectral analysis therefore revealed that there were harmonic variations superimposed on other harmonics and determination of the frequency of each harmonic was possible. Such oscillations have been observed by others (Veldhuis et al 1984, Filicori et al 1984), although statistical interpretations were not made. Their significance is unknown.

Sub-sampling of the 5 minute data produced 2 series of 10 minute data and 3 series of 15 minute data for analysis. Considering the dominant harmonic alone, inconsistent results were obtained with the 15 minute sampling interval but consistent results were observed between the two 10 minute series and between the 3 x 5 minute series. Accordingly, the failure of spectral analysis to detect harmonic variations in the PCOS study can be explained by the 15 minute sampling interval used.

FIGURE 6.4
SERIAL LH DATA AND PERIODOGRAM
(NORMAL SUBJECTS IN EARLY FOLLICULAR PHASE)

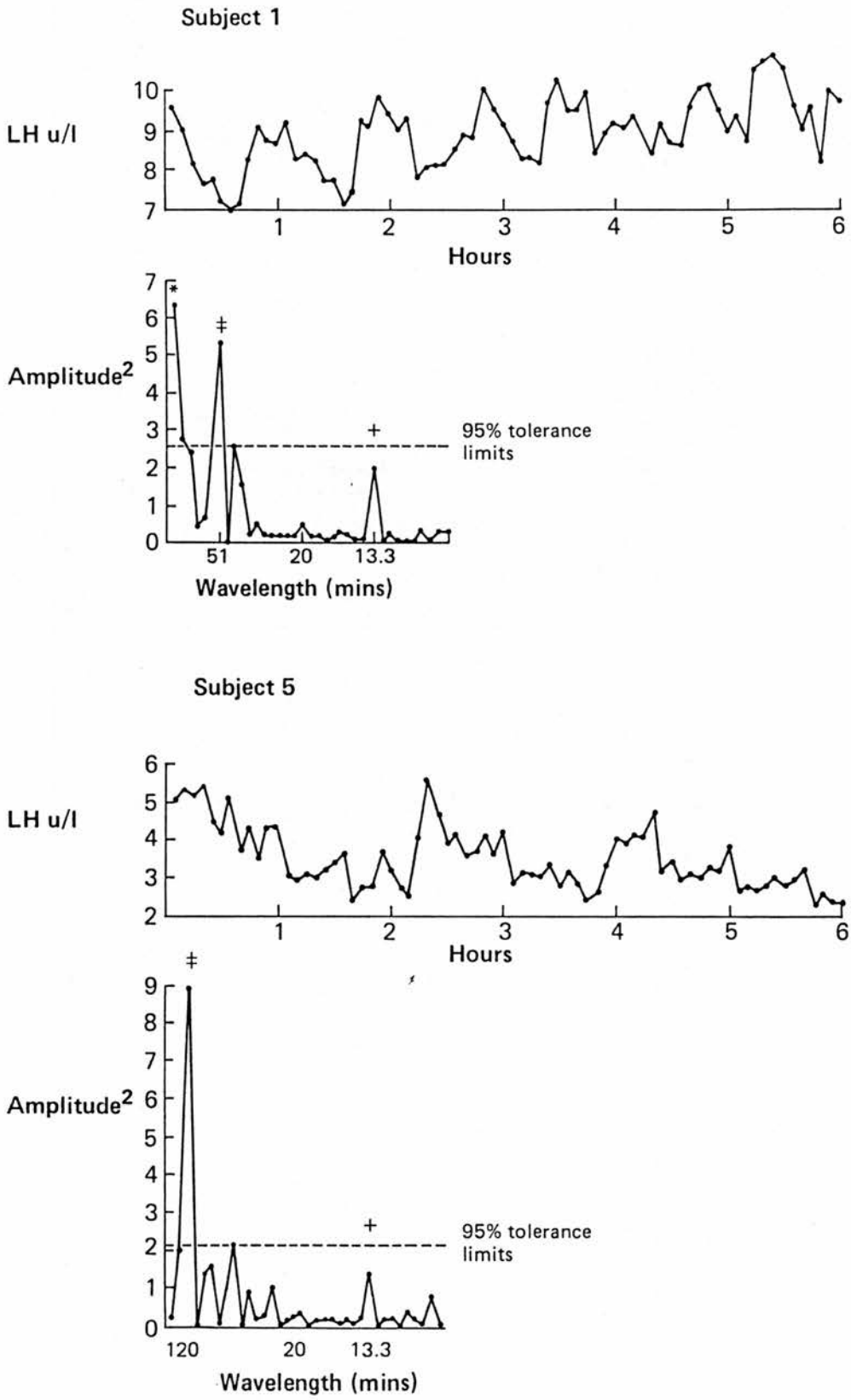
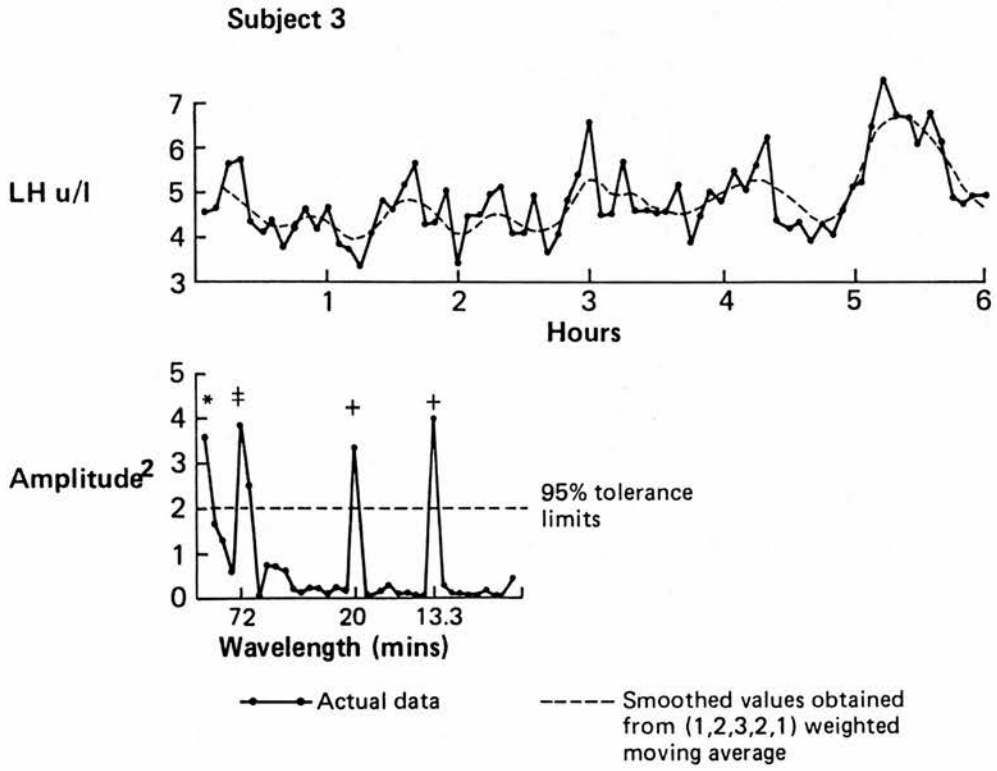


FIGURE 6.5

SERIAL LH DATA AND PERIODOGRAM
(NORMAL SUBJECT IN EARLY FOLLICULAR PHASE)



As a preliminary to further investigations, eight patients with PCOS were studied using 5 and 10 minute sampling intervals in order to determine the dominant harmonics. The results are given in Table 8.9 and the frequency and amplitude of the dominant periodogram ordinates are given in Table 6.5. Compared with the control group, there was an increase in frequency of the dominant harmonics. The absolute pulse amplitude was also increased and it therefore seemed likely that the elevated mean LH concenrations in PCOS are due to a combination of changes in pulse frequency and amplitude. As described previously, the latter may be a consequence of the hyperoestrogenic state in PCOS which directly sensitises the pituitary to LHRH. However, the increased pulse frequency offers further evidence for an aberration of hypothalamic function in PCOS.

TABLE 6.5

SPECTRAL ANALYSIS OF SERIAL LH DATA OBTAINED FROM 11 NORMAL WOMEN AND 8 PATIENTS WITH PCOS
(Data in Tables 8.8 & 8.9)

NORMAL WOMEN	Mean LH U/l	Frequency of dominant periodogram ordinate (min)	Amplitude U/l
5 minute sampling interval			
Subject 1	8.9	51	2.3
2	3.5	120	3.0
3	3.1	–	–
4	6.1	60	2.1
5	6.0	72	2.0
6	5.0	80	3.1
10 minute sampling interval			
Subject 7	2.4	120	1.7
8	5.6	150	3.0
9	2.9	220	1.0
10	6.7	96	4.1
11	4.0	120	2.8
PCOS			
5 minute sampling interval			
Subject 1	21.5	34	3.6
2	30.3	72	25.4
3	26.4	40	5.5
4	23.4	100	9.6
5	9.5	60	3.2
10 minute sampling interval			
Subject 6	16.1	60	4.8
7	13.4	55	3.2
8	10.1	120	4.2

CHAPTER 7: GENERAL DISCUSSION

7.1 SUMMARY OF CONCLUSIONS

The aim of this study has been to seek further evidence of a deficiency of DA in the hypothalamus in PCOS which could be part of the basic pathology of this condition. Such a deficency would be expected to alter prolactin secretion since this is under tonic inhibition from hypothalamic DA. The detailed studies of spontaneous prolactin secretion and tests of lactotroph reserve have failed to find a specific abnormality associated with PCOS. Furthermore, the observation that preceding ovarian function altered lactotroph responses may be the explanation for some of the previous studies which suggested that abnormal prolactin secretion was a feature of PCOS.

Dopamine agonist therapy given on a long term basis may be expected to relieve the symptoms of PCOS should such a deficency exist. Previous success with bromocriptine therapy in treating the symptoms of hirsutism in PCOS prompted a controlled trial to evaluate this treatment. No therapeutic value was found and no consistent biochemical improvements were observed. An increase in menstrual frequency occurred in both groups suggesting that this was a placebo effect.

Anticipating that the DA deficency was the origin of the typically elevated LH concentrations in PCOS, the latter was studied in detail before and during therapy with bromocriptine. No change was found in mean LH concentrations or in the amplitude and frequency of the LH pulses. The observation was consistent with the lack of clinical improvement found in this study.

In conclusion, these studies have failed to provide evidence which would support the hypothesis of a deficency of hypothalamic DA as a cause of PCOS.

7.2 FURTHER STUDIES INDICATED

A clue to the origins of PCOS lies in the observation that the syndrome starts at puberty. Although no longitudinal studies have been reported, it is the general experience which is confirmed by the histories of the patients studied here, that the events occurring around puberty are crucial. In childhood the hypothalamic-pituitary axis is highly sensitive to the feedback effects of oestrogen. At puberty, there is a rise in gonadotrophin secretion, largely due to a nocturnal increase in pulse amplitude and frequency (Boyar et al 1972). This reflects a primary hypothalamic event since it has been observed in agonadal subjects (Conte et al 1975). As the ovary responds and secretes oestrogen, there is a decrease in sensitivity of the hypothalamic-pituitary axis to feedback inhibition although the precise mechanism by which this occurs is unknown (Johnson and Everitt 1980). The balance between the ovary and the hypothalamic-pituitary axis appears to be "set" at this time. Thereafter it seems that each individual may retain her own pattern of LH pulsatile secretion from one cycle to the next (Murdoch et al 1986). It is possible that an abnormal threshold of feedback regulation may originate at puberty resulting in PCOS. Once set, this pattern is retained despite intermittent ovulation, spontaneous or induced. In order to investigate this hypothesis, much more information on the control of puberty is needed and therafter a prospective study could evaluate the factors which trigger abnormal feedback regulation in PCOS.

A more precise knowledge of the hypothalamic-pituitary defect in this condition may lead to an effective therapy. A possible hypothalamic deficiency of DA has been explored in this study but was not proven. However, evidence has been obtained which agrees with the concept that there is a hypothalamic problem in these patients. The LH pulse generator is at the hypothalamic level and this study has found that pulse frequency may be increased. This may be a primary problem or may result from altered feedback control.

Abnormal ovarian function in PCOS results in alteration of the usual feedback effects on the hypothalamus. Progesterone is the single unequivocal factor that has been found to change LH pulse frequency. In the normal cycle, pulses in the luteal (progesterone dominant) phase are slowed down to every 4 to 6 hours (Santen and Bardin 1973). Amenorrhoeic and oligomenorrhoeic women do not experience cyclical inhibition by progesterone and it is possible that under such circumstances the pulse generator operates more rapidly than normal. Contradicting this hypothesis is the lack of evidence to show that regular ovulation induction and hence cyclic endogenous progesterone secretion corrects the condition in the long-term.

The effect of oestrogens on LH pulses is less obvious. An increase in frequency probably occurs during folliculogenesis (Backstrom et al 1982) as oestradiol concentrations rise. In patients with PCOS, oestradiol concentrations are within normal limits although oestrone concentrations are elevated. The effect of the latter on LH pulses is unknown but may be the cause of the increase in frequency.

Oestradiol and progesterone are not the only hormones produced by the ovaries in response to stimulation. The ovarian androgens, androstenedione and testosterone, may have an effect on LH pulse frequency in women, although paradoxically testosterone slows pulse frequency in male and female monkeys (Plant 1986) and hence their importance is probably insignificant. Many other ovarian factors are now being discovered e.g. inhibin, whose role in the feedback regulation of LH is unknown. Any of these may be altered in PCOS and may be the source of the problem.

An alternative explanation for the increased LH pulse frequency is the possibility of an inherent abnormality of the LHRH pulse generator. As was discussed in Section 1.1.2, DA is not the only neurotransmittor which may be involved in LHRH regulation. Any of these other factors may be altered. In addition, the observation in monkeys of a close correlation between the LH pulses and hypothalamic multiunit electrical activity (Wilson et al 1984) suggests possible higher neuronal regulating factors. These too may be implicated in the pathophysiology of PCOS and warrant further investigation.

Another area which deserves more consideration is the possible existence of multiple pulses of LH. This suggests that its secretory pattern is much more complicated than had been previously appreciated. The physiological significance of these pulses is uncertain. There are distinct actions of LHRH on the gonadotroph, i.e. promotion of LH synthesis, storage and secretion (see Chapter 1). These have been demonstrated to be related to the dose and duration of LHRH stimulation. Therefore, every LHRH

pulse may have a separate physiological function. Several hypothalamic sites have been shown to contain LHRH secreting neurons. Possibly each secretes pulses of LHRH independently which would then be reflected by multiple LH pulses. Considering the numerous neurotransmitters found in the hypothalamus, separate regulating factors may influence each individual pulse.

Further studies are therefore needed initially to confirm the existence of these multiple pulses and thence to determine their normal controlling factors. Subsequently, interpretation of studies of the complex LH pulse patterns in patients with PCOS may lead to a better understanding of the precise hypothalamic pathology of this condition. The pathophysiology may then be revealed and a cure found.

'O never, never let us doubt
What nobody is sure about.'

(Belloc).

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TABLE 8.1A: PROLACTIN RESULTS OF MULTIPLE SAMPLING STUDY - POLYCYSTIC OVARY SYNDROME PATIENTS (mU/l)

Samples taken every 15 minutes for 6 hours from 08:30h

SUBJECT	1	2	3	6	7	9	12	14	16	18	23	26	28	33	37	39	40	41	42	43	48	49	50	51	52	53	54	58	61
	156	533	148	140	166	296	494	456	364	320	224	368	546	217	269	434	223	328	1052	277	313	280	322	304	271	416	216	375	467
	108	395	122	182	<100	283	286	449	318	265	209	352	550	217	243	444	204	281	1034	333	250	272	260	270	238	342	165	272	345
	135	474	127	189	<100	282	357	360	328	232	169	274	506	246	196	356	140	301	803	322	252	260	257	263	224	246	188	292	310
	127	391	<100	176	<100	253	265	293	269	248	141	217	459	210	221	339	172	221	671	287	219	207	243	237	184	207	126	223	202
	156	356	<100	181	<100	239	221	333	280	224	167	209	372	186	133	306	172	256	592	246	199	208	237	256	194	-	123	208	225
	137	336	125	172	<100	258	213	304	252	225	138	192	328	168	122	242	137	247	571	219	213	168	266	266	131	213	<100	195	207
	205	300	<100	166	<100	223	226	314	224	264	129	168	338	217	199	286	166	243	543	232	203	126	212	208	139	191	119	197	162
	135	322	136	211	<100	230	193	314	225	203	189	177	329	249	118	256	173	173	499	374	211	194	241	217	140	102	105	167	165
	125	363	<100	180	<100	211	142	308	152	189	152	143	264	215	<100	196	119	250	490	599	200	<100	239	229	147	125	149	<100	112
	129	330	<100	179	<100	239	151	326	185	222	162	159	304	213	178	166	140	207	467	659	198	<100	239	223	108	177	<100	<100	<100
	156	313	183	201	<100	259	146	284	161	183	125	148	341	190	168	149	113	196	455	579	193	117	385	253	164	<100	<100	187	151
	152	326	123	132	<100	205	128	291	142	192	126	133	192	197	187	143	110	263	417	486	206	<100	320	218	<100	<100	<100	137	<100
	173	301	101	160	<100	223	113	277	174	201	142	145	209	194	123	142	120	234	518	368	203	<100	317	231	<100	<100	<100	100	<100
	117	351	171	115	<100	243	<100	270	137	202	108	122	201	137	174	127	139	181	483	270	173	<100	407	223	<100	<100	<100	166	<100
	184	271	132	146	<100	212	<100	314	114	237	-	130	247	185	178	174	201	142	441	235	198	<100	424	227	121	136	<100	<100	145
	128	343	<100	178	<100	272	<100	281	166	214	161	159	304	233	159	201	207	<100	400	198	199	118	320	233	<100	<100	<100	<100	208
	137	360	<100	194	<100	276	163	299	147	233	117	129	227	177	172	143	164	<100	415	227	210	<100	425	238	138	<100	100	-	<100
	130	347	<100	217	<100	277	111	293	175	236	155	178	205	166	126	144	127	102	467	246	223	236	586	343	101	<100	<100	<100	<100
	144	257	125	186	114	336	149	231	171	183	145	159	193	204	136	162	139	<100	447	213	212	279	681	420	135	<100	<100	<100	<100
	<100	231	115	146	197	326	<100	386	190	239	198	215	189	245	185	167	206	135	430	179	247	229	541	314	133	122	<100	<100	<100
	<100	248	<100	178	439	350	<100	362	182	264	232	208	199	241	110	138	180	197	430	180	205	225	543	298	203	<100	151	<100	142
	<100	420	154	176	442	464	179	338	179	241	207	185	193	224	108	126	157	155	395	160	194	265	434	324	138	105	<100	<100	124
	121	354	<100	163	426	483	<100	363	172	230	192	156	207	221	143	215	199	187	431	197	166	243	399	298	133	161	172	<100	118
	105	414	147	144	384	375	123	345	173	178	212	177	264	223	184	236	189	181	449	214	190	285	341	469	114	125	154	123	152
MEAN	136	347	121	172	162	384	181	324	203	226	165	188	299	207	167	221	162	199	538	304	212	184	360	273	140	158	124	158	168

TABLE 8.1B: PROLACTIN RESULTS OF MULTIPLE SAMPLING STUDY - CONTROL DATA (mU/l)
Samples taken every 15 minutes for 6 hours from 08:30h

SUBJECT	1	2	3	4	5	6	7	8	9	10
	371	217	214	201	212	548	476	257	118	355
	390	190	177	151	186	439	311	227	<100	371
	381	174	174	148	152	307	278	219	121	382
	296	169	183	112	175	292	263	192	<100	399
	335	170	194	149	164	218	256	193	<100	329
	288	153	187	<100	151	210	223	192	<100	289
	-	151	136	<100	132	-	184	171	<100	286
	271	133	186	107	172	199	171	157	<100	234
	239	<100	189	<100	179	182	181	195	<100	234
	236	142	147	<100	180	174	<100	165	<100	229
	229	158	192	143	139	181	170	141	<100	212
	231	141	136	125	148	133	143	123	<100	246
	188	159	149	103	153	182	150	176	<100	224
	213	157	140	106	169	142	125	179	<100	-
	175	149	162	213	<100	153	150	140	<100	221
	171	139	143	267	147	183	193	140	<100	218
	222	157	152	272	131	185	135	159	<100	355
	202	168	-	233	120	140	166	155	<100	444
	224	174	163	259	<100	172	166	173	<100	423
	250	174	120	244	153	215	<100	183	<100	439
	219	149	154	122	<100	213	185	191	<100	368
	200	140	-	105	<100	182	202	<100	<100	360
	215	-	-	186	<100	160	174	135	<100	303
	205	-	-	184	<100	148	201	138	109	302
MEAN	250	148	165	156	141	213	195	171	102	314

TABLE 8.2: OVERNIGHT PROLACTIN CONCENTRATIONS (πU/l)
(hourly blood sampling)

TIME (h)	POLYCYSTIC OVARY SYNDROME PATIENTS					
1900	288	322	279	340		
	209	136	344	150		
	135	190	320	203		
	153	275	318	190		
2400	164	288	256	210		
	264	423	327	350		
	389	277	344	374		
	577	237	595	411		
0400	752	292	524	620		
	943	402	735	732		
	826	508	617	515		
	1006	487	642	436		
	492	396	652	390		
1000	310	200	280	197		
	CONTROLS					
1900	235	255	100	850	325	325
	125	340	100	440	265	175
	185	275	230	310	295	240
	190	380	245	485	330	320
	175	250	250	660	240	400
2400	205	320	240	425	245	470
	200	310	190	350	245	700
	-	215	295	665	245	700
	410	415	325	575	370	690
0400	335	420	285	440	480	685
	535	320	385	635	500	685
	575	-	475	595	525	600
	410	415	625	-	425	475
	425	535	295	-	365	350
	175	305	175	400	305	255
1000	100	185	125	235	195	190

TABLE 8.3A: SERUM PROLACTIN (mU/l) RESPONSES TO DOMPERIDONE, TRH, LHRH AND INSULIN HYPOGLYCAEMIA IN PATIENTS WITH PCOS (increments above basal value)

O: Studied in early follicular phase after ovulatory cycle
A: Studied after three months amenorrhoea

	DOMPERIDONE						
	Basal	15'	30'	45'	60'	90'	120'
A	418	2403	2911	2331	1799	1403	1151
A	203	2406	2350	2350	1880	1778	1430
A	351	3974	3678	3298	2838	2286	1810
A	405	1956	2848	3036	1876	1488	1252
O	357	5040	5844	5104	3898	2788	2144
A	246	3631	4195	3555	2803	2139	1827
A	394	2530	2574	2290	1978	1242	1026
O	194	2987	3903	3503	2627	2051	1659
A	398	1862	2392	2202	1657	1287	1077
O	252	5628	5418	4513	3508	2818	2603
A	438	1912	2077	1457	1302	872	667
O	469	4626	6621	6676	6316	5641	5301
O	175	3845	4095	2855	2610	2110	1800
A	285	3300	4380	3455	2680	2140	1870
A	203	1952	2227	1472	1197	1092	777
A	331	2504	2809	2309	-	1329	1164
O	205	4215	3640	2625	2690	2205	1470

	LHRH			
	Basal	20'	40'	60'
A	278	71	36	103
O	184	90	56	60
	142	6	0	-
	118	83	78	36
A	151	65	105	90
O	164	28	25	35
A	207	-30	-51	-50
	110	15	95	70
A	138	42	93	93
O	211	83	87	72
A	420	-106	-141	-137
A	142	27	191	212
A	345	102	175	106
A	100	124	121	95
O	431	12	-70	-171
O	187	133	83	64
	191	63	73	89
A	286	77	102	170
	256	67	113	142
A	100	86	85	10
A	124	89	156	179
	111	96	151	56
A	100	168	220	275
	403	403	418	-27

	TRH		
	Basal	20'	60'
A	205	737	199
A	254	1501	266
O	527	1973	1185
O	169	1126	572
O	100	1129	394
O	551	1949	561
A	297	843	418
A	169	1070	351
A	149	632	161
A	178	712	197
A	243	512	82

	INSULIN HYPOGLYCAEMIA				
	BASAL	30'	60'	90'	120'
A	200	15	235	45	-35
A	100	0	680	315	120
A	280	-20	935	505	285
O	400	535	2105	1200	775
A	380	310	1200	250	5
A	125	15	330	215	100
A	270	50	435	245	120

8.3B: SERUM PROLACTIN (mU/l) RESPONSES TO DOMPERIDONE, TRH, LHRH AND INSULIN HYPOGLYCAEMIA IN CONTROL WOMEN (increments above basal values)

DOMPERIDONE							LHRH				TRH			INSULIN HYPOGLYCAEMIA				
Basal	15'	30'	45'	60'	90'	120'	Basal	20'	40'	60'	Basal	20'	60'	Basal	30'	60'	90'	120'
180	5135	6080	5660	5305	3560	2950	184	185	249	201	966	3879	1019	241	21	1084	534	344
550	4770	4945	4555	4215	3595	2800	161	530	695	446	315	1875	865	191	-31	1504	989	474
833	6832	9027	7677	5177	4302	3752	128	82	118	67	418	2122	947	221	571	1264	754	374
221	4179	4529	2604	3554	2439	2164	172	1	15	-16	265	1040	435	282	25	1104	868	518
261	5239	5729	5874	5279	3819	2779	99	77	63	82	263	1352	637	272	94	1563	1108	668
312	5468	5468	4613	1313	2993	2698	181	203	250	237	193	857	407	223	-26	187	307	92
547	3973	4188	3878	3033	2478	1853	212	194	369	299	270	1520	685	320	-57	630	255	250
115	2800	4180	3550	2885	2435	2260	184	40	70	22	306	1259	344					
244	2761	3456	2916	2721	1956	696	351	53	50	3								
404	3751	4956	3691	3226	2556	2151	100	120	134	94								
226	4134	5039	4054	3489	2744	2374												
323	5007	5707	4352	3867	2912	2372												
475	2340	3010	2280	2190	2210	1865												

TABLE 8.4: INDIVIDUAL RESULTS OF NUMBER OF HAIRS GROWING (area 24 x 36 mm) DURING TRIAL

SUBJECT	FACE			ABDOMEN		
	Months of treatment			Months of treatment		
	0	6	12	0	6	12
BROMOCRIPTINE GROUP						
42	76	76	76	36	20	23
16	38	34	33	24	14	14
39	96	85	41	28	24	16
46	26	38	16	41	49	43
49	33	33	33	38	38	42
41	50	48	56	13	16	13
1	4	2	4	13	13	12
PLACEBO GROUP						
33	74	59	67	25	25	29
28	2	26	25	61	57	71
36	30	30	30	36	26	34
23	21	18	20	32	28	29
37	21	8	28	45	-	28
52	37	26	41	26	30	30
57	7	1	2	27	30	33
48	6	8	1	5	7	7
58	30	16	17	14	16	22

TABLE 8.5: INDIVIDUAL RESULTS OF HAIR GROWTH RATES (mm/day) DURING TRIAL

BROMOCRIPTINE GROUP						
SUBJECT	FACE			ABDOMEN		
	Months of treatment			Months of treatment		
	0	6	12	0	6	12
42	.36	.39	.34	.36	.34	.34
16	.39	.42	.36	.29	.36	.31
39	.31	.26	.21	.34	.39	.29
46	.21	.18	.21	.29	.18	.23
49	.21	.16	.10	.23	.29	.29
41	.42	.39	.23	.18	.39	.21
1	.21	.18	.18	.18	.21	.18
PLACEBO GROUP						
33	.36	.36	.36	.29	.31	.39
28	.34	.31	.34	.36	.36	.31
36	.31	.26	.34	.21	.23	.31
23	.31	.29	.29	.39	.36	.26
37	.26	.23	.23	.34	-	.26
52	.26	.31	.26	.29	.29	.31
57	.18	.21	.26	.29	.42	.44
48	.23	.39	.31	.34	.29	.23
58	.34	.23	.29	.26	.29	.29

TABLE 8.6A: GONADOTROPHIN CONCENTRATIONS DURING TRIAL: MULTIPLE SAMPLING STUDY AND RESPONSE TO LHRH (100 ug IV)
- BROMOCRIPTINE GROUP

SUBJECT		49			42			39			1			41			16		
MONTHS OF THERAPY		0	6	12	0	6	12	0	6	12*	0	6	12	0	6	12	0	6	12
1		12.5	6.1	14.0	7.9	5.8	6.3	9.7	10.4	53.8	12.5	18.5	14.8	19.0	18.2	25.9	6.7	3.0	1.9
2		13.2	6.5	13.0	6.6	5.7	6.2	13.6	10.7	48.6	13.4	14.7	14.9	19.2	20.6	22.9	6.5	3.0	2.8
3		14.9	7.1	12.8	5.3	5.0	5.6	14.4	15.8	40.0	12.8	14.0	13.4	20.9	19.1	23.4	5.5	1.2	2.2
4		13.5	6.7	13.1	4.2	4.8	4.6	15.1	14.5	55.8	12.1	10.9	12.2	23.2	17.4	24.5	4.8	1.0	1.6
5		16.3	5.9	12.2	5.3	4.3	5.2	15.9	11.6	52.1	11.1	14.6	11.6	16.4	23.0	22.5	6.0	2.0	1.4
6		13.5	5.1	11.3	5.5	6.1	4.6	14.5	11.1	39.5	9.3	14.6	16.3	25.9	22.5	21.5	6.7	2.6	2.1
7		12.2	5.0	13.3	4.7	4.8	4.5	15.4	10.2	59.4	12.6	12.6	13.2	23.8	18.8	19.2	6.8	1.4	2.0
8		15.8	4.5	13.9	6.0	4.1	4.1	13.6	13.8	46.8	10.8	11.0	15.3	20.7	21.2	19.4	5.9	2.1	1.6
9		13.9	7.4	13.1	6.1	4.7	3.9	11.7	12.9	35.4	11.4	17.5	14.5	16.3	23.9	25.6	8.9	2.2	3.6
10		13.5	9.6	10.9	6.5	5.5	4.3	10.0	11.6	30.5	12.8	13.9	13.0	22.7	24.8	25.6	8.9	2.2	3.6
11		18.9	7.4	10.5	5.3	5.8	6.3	9.2	9.9	81.5	10.7	13.0	12.9	18.8	21.1	17.2	7.0	2.7	2.7
12		15.1	6.5	11.7	5.4	4.2	5.6	7.8	9.4	57.5	12.1	13.8	13.7	24.5	23.4	22.5	7.2	2.6	1.9
13		15.8	6.1	14.5	7.3	5.3	4.6	7.8	12.1	36.5	11.8	16.5	13.9	26.5	26.1	23.0	6.3	1.7	2.9
14		13.5	5.5	14.4	5.7	5.0	4.6	7.4	16.4	32.8	10.0	15.0	13.5	16.3	21.8	25.3	5.9	4.1	2.8
15		15.0	5.6	11.2	5.7	3.5	5.1	13.1	14.2	57.0	11.3	12.5	14.0	-	18.9	25.2	4.7	2.7	1.8
16		14.9	5.8	11.3	5.0	3.6	4.3	11.3	11.8	49.1	13.1	17.8	18.5	-	16.0	22.0	7.7	2.5	2.8
17		13.5	11.2	13.0	6.4	4.8	4.1	10.9	10.4	42.0	10.7	16.2	21.2	-	15.5	25.6	7.1	-	2.5
18		15.2	7.1	11.5	6.6	5.2	4.8	9.0	8.3	44.4	14.4	13.5	20.9	-	26.6	23.3	7.9	-	3.1
19		14.7	6.0	11.0	4.8	4.2	6.3	8.5	15.0	70.5	12.3	15.7	16.9	-	25.5	19.5	5.7	27	1.4
20		19.6	5.5	14.8	4.8	4.2	6.4	7.7	17.7	49.1	11.8	14.1	15.0	-	23.2	18.0	5.3	27	1.6
21		14.1	5.6	18.3	4.4	3.3	5.0	7.1	14.3	51.9	13.3	12.9	13.4	19.6	22.7	24.4	7.5	19	2.8
22		14.8	14.3	6.5	6.5	3.5	4.7	7.9	11.7	55.4	11.5	13.6	22.1	15.5	29.9	20.0	5.9	28	2.2
23		13.8	8.8	12.9	5.6	7.1	4.3	12.6	10.8	33.7	11.7	16.5	-	18.4	23.8	16.5	5.6	27	2.9
24		16.4	8.4	12.3	5.9	6.2	3.7	11.9	9.5	32.5	12.2	4.3	6.7	23.2	21.6	17.5	5.6	24	2.5
Mean LH		14.8	7.0	12.8	5.7	4.9	5.0	11.1	12.3	48.2	11.9	14.5	15.3	20.6	21.9	22.1	6.4	2.4	2.3
No. of pulses		3	4	4	4	3	2	3	4	4	0	5	3	3	4	3	4	3	3
Mean amplitude		3.4	2.9	5.1	1.5	2.2	2.2	4.2	5.7	24.9	-	3.7	3.4	4.4	6.6	4.6	2.3	1.5	1.2
LH response to LHRH	0'	14.7	-	4.6	7.2	3.2	3.7	10.7	8.7	48.7	12.1	14.4	13.0	13.7	24.0	19.7	7.3	3.5	2.2
	20'	39.2	-	7.5	11.4	4.6	4.8	24.4	25.4	>250	49.8	50.1	48.0	57.0	42.8	48.2	25.6	13.0	8.1
	40'	60.4	-	7.7	13.8	4.0	6.6	-	-	>250	90.6	-	47.8	83.8	64.0	59.2	23.0	14.4	-
	60'	60.0	-	8.1	12.0	4.4	6.2	-	38.4	>250	74.4	63.1	59.8	78.0	81.8	59.0	23.7	11.5	8.3
FSH u/l (mean of 6 samples)		4.9	3.8	3.4	3.1	2.7	3.5	4.7	3.6	4.3	3.5	3.2	4.1	3.4	3.8	3.9	4.5	3.4	3.4
FSH response to LHRH	0'	6.5	-	4.6	3.3	3.2	3.7	4.0	4.5	4.2	6.0	3.3	4.1	3.4	3.8	3.9	4.5	3.4	3.4
	20'	8.2	-	7.5	5.5	4.6	4.8	7.1	6.4	8.9	8.2	5.3	8.6	5.5	5.4	5.8	8.3	5.6	4.7
	40'	9.3	-	7.7	7.4	4.0	6.6	-	5.2	9.0	7.4	-	9.0	6.9	7.4	6.3	8.1	7.9	-
	60'	9.6	-	8.1	4.9	4.4	6.2	-	3.8	8.6	4.8	7.2	4.0	6.1	8.0	6.9	7.6	7.6	6.2

TABLE 8.6B: GONADOTROPHIN CONCENTRATIONS DURING TRIAL: MULTIPLE SAMPLING STUDY AND RESPONSE TO LHRH (100 ug IV
- PLACEBO GROUP

SUBJECT		28			33			23			58			37			48		
MONTHS OF THERAPY		0	6	12	0	6	12	0	6	12	0	6	12	0	6	12	0	6	12
1		13.8	13.0	12.6	4.2	4.7	2.7	12.0	8.0	11.6	12.0	15.6	13.3	7.0	9.4	8.0	5.5	7.1	5.5
2		14.3	11.6	11.2	5.1	4.2	4.4	11.0	9.2	10.4	12.2	12.0	12.5	5.8	9.0	10.0	5.8	4.1	6.5
3		16.5	12.5	10.8	5.8	4.5	-	12.9	8.8	9.0	14.0	22.4	13.9	7.1	6.9	8.8	5.0	4.0	3.8
4		13.0	12.6	10.5	5.3	3.1	-	11.6	6.6	11.0	13.4	17.8	13.3	8.3	5.8	7.9	4.9	3.2	3.6
5		10.8	10.4	8.8	5.4	3.0	4.6	9.9	5.7	12.1	10.9	14.5	12.7	8.4	5.5	6.9	4.8	3.8	3.4
6		11.1	13.4	13.5	4.6	3.3	4.1	8.8	9.3	11.9	12.7	13.1	13.6	6.7	6.2	8.8	4.6	3.9	4.0
7		12.9	12.3	11.3	6.1	5.7	4.3	10.5	10.6	11.9	12.5	16.1	12.5	5.7	8.6	8.1	5.1	4.8	4.0
8		11.5	10.9	9.7	4.8	5.3	4.6	12.5	8.3	11.3	11.3	15.9	11.7	4.4	8.2	7.2	6.3	5.3	4.6
9		10.7	12.6	13.7	4.7	5.1	4.1	11.0	8.1	10.7	9.8	14.6	13.8	7.9	8.0	6.8	4.3	4.1	4.9
10		14.4	14.6	11.1	5.2	4.9	5.2	13.5	8.6	11.2	9.1	14.2	12.7	7.3	6.2	9.3	4.3	4.1	4.5
11		13.0	11.6	13.7	5.6	4.5	4.1	13.1	9.5	11.9	12.9	18.1	12.6	6.4	5.5	8.8	2.9	4.1	5.0
12		10.5	10.9	14.3	6.0	4.4	4.1	10.5	7.8	11.1	12.4	14.2	15.1	7.4	4.6	7.8	5.7	5.0	3.9
13		15.6	14.5	12.5	5.5	5.5	4.1	10.8	9.6	10.2	11.4	13.9	14.4	7.7	4.6	9.9	5.7	4.4	3.9
14		16.2	10.4	15.4	5.1	5.7	4.0	11.9	10.4	9.4	13.4	12.6	13.4	8.8	4.5	9.2	-	5.4	4.3
15		14.5	15.8	10.4	6.0	4.6	6.0	11.7	7.6	9.2	13.6	14.9	14.3	9.3	9.0	7.8	5.8	10.7	3.9
16		15.6	14.0	11.9	5.4	4.3	4.3	12.6	8.0	11.4	11.6	13.4	13.5	9.2	8.3	7.2	5.4	11.6	5.5
17		12.1	12.4	17.6	5.4	3.6	3.9	12.7	8.5	11.2	10.4	11.8	12.4	8.2	6.4	6.4	7.1	10.0	3.7
18		11.9	19.6	13.2	4.3	3.8	4.2	12.7	8.5	10.2	15.5	15.0	11.5	6.7	5.4	9.7	6.4	7.4	4.0
19		16.0	-	12.0	5.1	6.4	2.9	12.4	8.7	9.7	14.7	14.6	13.1	7.3	4.7	7.3	8.0	7.4	4.1
20		13.3	14.3	14.8	6.2	5.1	4.0	13.4	7.4	10.5	11.7	13.8	15.2	9.0	4.4	9.3	6.3	6.9	4.7
21		15.1	13.9	14.6	5.5	4.5	5.9	11.8	9.8	10.7	10.4	13.7	12.3	6.8	4.7	7.3	8.0	7.4	4.1
22		14.3	11.3	12.0	5.5	4.5	5.4	12.2	9.6	8.6	13.5	19.8	11.0	4.6	4.0	6.6	7.5	7.4	3.3
23		13.3	20.8	10.6	5.5	3.7	5.6	11.4	8.7	9.0	12.0	15.7	14.1	6.1	6.6	9.4	5.2	5.4	8.9
24											11.2	14.4	13.8	6.6	8.0	8.5	5.2	5.2	7.3
Mean LH		13.5	13.5	12.5	5.4	4.5	4.4	11.7	8.6	10.5	12.2	15.1	13.2	7.2	6.4	8.3	5.5	6.0	4.6
No. of pulses		4	2	3	3	3	2	4	5	3	5	5	4	4	4	3	5	3	4
Mean amplitude		3.4	5.1	4.2	1.2	2.1	2.4	1.7	1.9	1.6	3.2	4.8	2.5	1.8	3.6	2.2	1.8	2.8	1.8
LH response to LHRH	0'	15.4	13.2	19.2	5.7	4.2	4.3	12.3	7.9	7.3	8.9	14.4	13.4	6.6	7.1	7.7	4.7	5.2	7.3
	20'	61.6	117.2	57.6	15.9	11.3	14.7	32.0	31.8	37.1	38.2	52.2	40.0	18.2	12.3	16.2	4.8	49.6	16.2
	40'	91.2	104.8	88.8	19.8	12.0	14.7	29.2	32.6	-	49.6	57.0	33.2	23.4	12.2	18.0	5.6	33.9	-
	60'	71.0	117.4	92.8	16.2	11.1	12.1	23.6	28.2	45.8	46.4	54.2	43.8	12.4	11.8	14.5	4.9	22.1	11.7
FSH u/l (mean of 6 samples)		4.3	3.7	3.9	3.2	3.2	3.0	3.8	3.3	4.2	4.2	4.1	3.4	3.5	4.2	4.2	4.0	2.4	4.3
FSH response to LHRH	0'	4.6	3.7	3.9	5.7	4.2	3.4	5.2	4.4	4.7	4.4	4.6	5.2	4.5	4.9	5.7	-	2.2	4.7
	20'	6.8	4.6	6.0	15.9	11.3	5.5	5.3	6.7	8.5	7.5	7.7	5.7	6.6	5.8	7.4	-	8.5	7.7
	40'	7.6	8.6	8.2	19.8	12.0	7.0	8.6	7.9	-	6.7	8.4	6.7	7.7	4.7	8.4	-	9.0	-
	60'	7.9	7.3	8.8	16.2	11.1	6.7	6.0	5.2	9.1	7.1	8.4	6.7	6.9	6.9	9.1	-	8.5	4.7

TABLE 8.6C: GONADOTROPHIN CONCENTRATIONS DURING TRIAL: MULTIPLE SAMPLING STUDY AND RESPONSE TO LHRH (100 ug IV) - PLACEBO GROUP (cont) (Defaulters = 1 study only)

SUBJECT		52			18		43	7	51	50
MONTHS OF THERAPY		0	6	12	0	6				
1		12.1	10.1	8.5	10.9	9.7	8.0	8.4	9.4	10.8
2		13.7	8.7	9.4	8.3	11.0	6.7	7.3	9.6	8.7
3		11.3	7.0	6.9	9.2	11.0	6.4	9.6	8.0	6.0
4		10.9	8.6	6.1	8.8	11.0	6.9	9.2	13.1	5.5
5		9.2	9.3	5.9	8.2	11.6	6.6	7.5	10.2	5.1
6		8.0	8.0	8.4	10.8	9.8	6.2	7.5	8.8	7.1
7		9.6	7.8	8.3	9.0	9.1	5.9	9.5	7.6	6.9
8		13.0	6.7	6.2	8.3	9.4	5.6	9.6	11.5	6.2
9		12.9	6.4	6.1	8.9	11.1	5.8	8.7	12.7	6.8
10		9.8	5.7	5.3	7.3	13.0	5.7	10.2	8.7	5.2
11		11.2	5.6	6.8	7.7	9.0	5.8	10.8	7.6	6.6
12		11.0	4.6	6.2	9.4	9.2	5.2	9.6	14.3	7.7
13		9.9	9.2	6.1	10.2	11.7	5.2	7.5	10.2	5.7
14		9.6	8.3	6.0	10.2	10.2	6.0	8.4	10.3	6.1
15		8.9	6.8	6.0	9.0	10.8	6.0	7.5	10.8	5.8
16		8.9	5.9	5.8	8.5	12.6	6.3	11.4	10.1	5.9
17		9.6	5.8	5.8	12.1	11.4	7.3	9.7	9.6	6.0
18		8.2	7.5	4.6	10.6	10.8	7.1	13.2	13.2	5.6
19		4.6	6.6	6.4	10.3	10.3	6.6	12.4	10.4	5.4
20		7.8	6.6	6.3	7.5	11.5	5.8	9.9	8.0	5.3
21		7.8	6.0	6.3	11.0	11.8	5.6	12.3	9.4	6.1
22		7.1	5.7	5.7	10.8	10.1	5.2	11.6	12.3	6.3
23		6.5	6.2	6.6	9.0	9.5	4.1	9.0	10.9	5.9
24		5.9	5.4	-	11.5	14.4	5.4	7.8	10.0	5.1
Mean LH		9.5	7.1	6.5	9.3	10.8	6.1	9.5	10.3	6.3
No. of pulses		1	2	3	4	4	2	5	5	2
Mean amplitude		4.2	3.8	1.6	2.3	2.41	0.9	2.3	4.4	1.4
LH response to LHRH	0'	7.0	5.6	-	11.2	13.4	6.7	13.0	11.6	4.8
	20'	19.9	15.8	-	23.6	29.9	100.4	45.2	32.6	22.1
	40'	30.7	20.4	-	39.1	37.7	151.8	45.2	33.6	22.9
	60'	46.2	24.7	-	31.0	40.3	116.8	38.4	34.7	21.7
FSH u/l (mean of 6 samples)		5.3	5.8	4.7	4.0	4.5	2.7	4.7	4.4	4.0
FSH response to LHRH	0'	4.7	5.8	-	11.2	3.8	2.3	7.9	4.4	4.1
	20'	7.3	8.2	-	23.6	6.0	8.9	8.3	3.3	5.4
	40'	7.2	9.3	-	39.1	7.0	10.7	7.8	5.5	5.6
	60'	6.1	6.4	-	31.0	7.4	11.2	6.4	6.8	5.7

TABLE 8.7A: STEROID CONCENTRATIONS DURING TRIAL - BROMOCRIPTINE GROUP

Subject	Months of study	Testosterone (nmol/l)	Androstenedione (nmol/l)	Oestrone (pmol/l)	Oestradiol (pmol/l)	SHBG (nmol/l)
49	0	5.0	19.4	392	198	18
	6	3.1	7.1	260	126	21
	12	4.3	10.5	504	428	33
42	0	4.4	15.6	300	104	33
	6	2.6	9.0	170	76	36
	12	2.4	9.4	167	104	27
39	0	3.4	16.6	382	120	28
	6	3.9	11.5	338	129	42
	12*	4.8	19.3	632	67O	48
1	0	4.3	28.2	372	212	37
	6	3.2	14.2	449	357	57
	12	3.5	11.2	292	98	51
41	0	4.1	24.0	326	296	24
	6	3.1	13.2	306	197	28
	12	3.3	12.0	379	247	27
16	0	6.0	31.6	305	170	-
	6	3.7	10.8	283	105	34
	12	3.7	11.7	305	260	39
46	0	2.6	11.4	320	179	-
	6	3.0	5.9	253	65	-
	12	-	-	-	-	-

*Study coincided with mid-cycle surge.

TABLE 8.7B: STEROID CONCENTRATIONS DURING TRIAL - PLACEBO GROUP

PLACEBO GROUP						
Subject	Months of study	Testosterone (nmol/l)	Androstenedione (nmol/l)	Oestrone (pmol/l)	Oestradiol (pmol/l)	SHBG (nmol/l)
28	0	4.2	16.4	327	160	22
	6	-	15.3	-	188	38
	12	3.8	11.7	285	160	40
33	0	3.3	8.9	510	117	25
	6	2.4	21.5	400	81	27
	12	2.6	8.9	270	113	28
23	0	3.7	13.8	507	-	72
	6	2.3	5.0	274	120	80
	12	3.7	8.7	298	117	76
58	0	3.6	18.2	420	248	29
	6	3.4	13.8	332	201	31
	12	3.0	11.2	275	350	26
37	0	4.0	42.0	433	131	37
	6	2.8	12.2	335	-	67
	12	3.7	5.9	310	131	46
48	0	1.9	7.2	-	82	104
	6	2.3	4.9	110	52	98
	12	2.0	3.5	195	90	88
52	0	2.5	13.5	426	127	-
	6	3.4	7.7	390	197	16
	12	-	-	548	253	-
18	0	2.7	10.3	133	89	29
	6	2.2	7.8	168	171	36
57	0	2.6	9.8	360	174	20
	6	2.7	9.1	-	181	45
	12	3.1	12.0	560	179	24
36	0	3.8	16.8	380	145	27
	6	4.3	15.3	-	206	-
	12	3.2	10.0	337	166	23

TABLE 8.8A: SERIAL LH DATA (u/l) FROM NORMAL WOMEN SAMPLING IN THE EARLY FOLLICULAR PHASE OF THE MENSTRUAL CYCLE - SAMPLES 1-34

(5minute sampling interval subjects 1-6; 10 minute sampling interval subjects 7-11)

SUBJECT	1	2	3	4	5	6	7	8	9	10	11
1	9.6	7.9	4.7	5.4	5.1	5.8	6.9	2.2	2.8	7.0	4.1
2	9.0	7.8	4.8	4.6	5.4	6.0	6.7	2.2	2.4	6.4	3.4
3	8.1	7.3	5.8	5.9	5.2	5.6	6.5	2.3	2.2	6.4	3.8
4	7.6	7.8	5.9	7.3	5.5	5.5	7.8	2.0	2.0	6.0	3.6
5	7.7	5.9	4.5	6.4	4.5	5.1	7.4	1.6	1.8	5.4	2.7
6	7.1	5.2	4.2	6.3	4.2	5.2	5.7	1.4	1.8	5.1	3.0
7	6.9	6.8	4.5	8.2	5.1	4.6	5.6	1.8	1.9	4.8	2.4
8	7.0	6.4	3.8	5.7	3.7	4.4	5.4	2.2	3.6	4.8	2.4
9	8.3	6.8	4.2	6.5	4.4	4.6	5.3	2.9	3.7	5.9	2.4
10	9.2	6.9	4.7	5.8	3.5	3.9	4.8	2.6	3.6	5.4	2.0
11	8.8	7.0	4.2	6.1	4.4	3.5	4.5	2.4	-	4.7	4.2
12	8.7	7.4	4.8	7.0	4.4	3.5	4.2	2.1	2.7	5.0	6.7
13	9.3	5.8	3.9	5.5	3.1	3.3	4.1	3.0	2.3	4.9	5.4
14	8.2	5.9	3.8	4.7	2.9	2.8	3.9	2.5	2.1	4.7	6.2
15	8.4	5.3	3.4	4.9	3.1	2.8	5.8	2.7	1.9	4.4	5.6
16	8.2	5.1	4.1	4.5	3.0	3.5	8.8	2.2	1.9	6.5	4.7
17	7.7	5.5	5.0	4.6	3.2	2.4	7.5	2.4	1.8	5.9	3.4
18	7.0	5.1	4.7	4.2	3.4	2.6	7.0	2.7	1.9	5.2	3.2
19	7.0	5.5	5.1	4.9	3.7	3.3	6.9	3.1	2.8	5.2	3.4
20	7.3	4.8	5.8	4.0	2.4	3.3	6.1	2.5	3.0	6.0	3.8
21	9.3	4.5	4.4	4.2	2.8	2.7	5.1	2.4	3.5	6.0	7.4
22	9.1	4.8	4.4	6.6	2.8	2.4	5.0	2.3	3.3	5.8	6.9
23	9.9	5.8	5.2	7.9	3.8	2.5	5.2	2.4	2.9	5.1	3.6
24	9.4	7.1	3.5	7.4	1.8	2.4	4.8	1.9	2.9	5.9	-
25	9.0	6.8	4.5	7.0	2.7	2.2	4.7	3.3	2.4	5.9	5.2
26	9.4	6.2	4.5	6.4	2.5	2.5	4.3	3.8	2.3	5.5	4.4
27	7.8	6.1	5.0	6.5	4.1	2.8	6.1	3.7	2.1	5.2	4.0
28	8.1	7.2	5.2	7.1	5.6	2.0	7.2	3.5	1.8	4.9	3.7
29	8.1	5.1	4.1	5.6	4.7	1.8	6.2	3.1	1.8	5.1	3.4
30	8.1	5.2	4.1	6.1	3.9	2.9	5.7	2.7	1.9	6.9	-
31	8.5	5.2	5.0	6.8	4.3	1.8	5.4	4.1	1.5	7.8	2.9
32	8.9	4.8	3.6	5.4	3.6	1.8	5.7	4.0	1.5	7.1	2.6
33	8.7	5.2	4.0	5.6	3.8	1.9	5.0	3.4	1.4	6.3	4.5
34	10.1	4.9	4.9	5.1	4.2	2.2	4.6	3.2	1.4	6.2	3.8

TABLE 8.8B: SERIAL LH DATA (u/l) FROM NORMAL WOMEN SAMPLING IN THE EARLY FOLLICULAR PHASE OF THE MENSTRUAL CYCLE - SAMPLES 35-68

SUBJECT	1	2	3	4	5	6	7	8	9	10	11
35	9.5	5.5	5.5	5.2	3.7	1.8	5.4	3.7	2.8	6.9	3.8
36	9.2	7.5	6.7	5.7	4.2	2.2	5.8	3.6	3.8	-	4.8
37	8.7	5.8	4.5	4.9	2.9	2.9	6.0	4.1		5.9	
38	8.3	7.0	4.6	6.5	3.2	2.0	5.8	2.0		7.3	
39	8.3	5.6	5.8	7.2	3.1	1.8	5.4	4.6		6.4	
40	8.1	5.9	4.6	6.5	3.0	2.7	4.8	4.1		6.3	
41	9.7	5.9	4.6	7.1	3.4	3.9	4.7	4.1		6.0	
42	10.3	5.6	4.5	6.8	2.8	2.8	4.1	3.2		7.4	
43	9.5	5.6	4.6	6.3	3.2	2.7	4.4	2.9		7.3	5.9
44	9.5	7.2	5.2	7.1	2.9	2.4	4.3	2.7		6.8	5.3
45	10.0	6.2	3.9	5.8	2.4	2.4		3.0		6.1	6.5
46	8.4	6.4	4.6	5.4	2.6	2.9					
47	8.9	6.5	5.1	5.3	3.4	2.6		4.8		9.2	5.9
49	9.0		5.5		4.0	2.1		5.0		9.4	
51	8.9		5.5		4.1	3.4					
52	8.2		6.3		4.9	2.9					
53	9.1		4.4		3.2	2.8					
54	8.6		4.2		3.4	2.8					
55	8.6		4.3		3.0	3.3					
56	9.6		3.9		3.1	2.3					
57	10.1		4.3		3.0	2.3					
58	10.2		4.0		3.3	2.9					
59	9.2		4.5		3.2	2.3					
60	8.9		5.2		4.0	2.1					
61	9.4		5.4		2.7	2.2					
62	8.4		6.6		2.8	2.5					
63	10.6		7.6		2.7	1.7					
64	10.8		6.2		2.8	1.5					
65	10.9		6.2		3.1	2.6					
66	10.6		5.7		2.8	1.5					
67	9.6		5.8		3.0	2.1					
68	8.9		6.2		3.3	2.5					

TABLE 8.9A: SERIAL LH DATA (u/l) FROM PATIENTS WITH PCOS SAMPLING IN EARLY FOLLICULAR PHASE OF THE CYCLE WHERE APPROPRIATE - SAMPLES 1-36

(5 minute sampling interval subjects 1-5; 10 minute sampling interval subject 6-8)

SUBJECT	1	2	3	4	5	6	7	8
1	22.7	26.4	23.0	23.4	11.6	13.2	15.6	9.9
2	20.7	26.2	26.5	22.1	11.5	18.0	13.5	8.1
3	21.9	26.4	28.7	19.5	11.0	16.8	15.1	7.4
4	25.0	26.9	29.4	20.9	10.3	14.9	11.8	11.5
5	23.3	26.6	27.9	17.2	11.8	13.9	13.6	10.1
6	25.8	26.9	34.9	17.7	11.7	15.2	14.0	8.2
7	18.6	27.3	24.7	20.7	11.4	14.3	13.2	11.8
8	19.2	29.9	23.2	22.0	10.9	14.4	11.6	10.2
9	18.8	23.9	28.1	22.9	10.3	21.5	11.3	8.4
10	18.1	22.0	27.1	22.5	9.5	20.5	14.4	7.5
11	19.0	22.8	26.7	21.4	9.2	16.9	13.8	11.7
12	23.0	27.3	24.8	21.0	8.9	16.3	12.6	9.5
13	18.8	25.2	26.4	21.8	8.1	14.0	10.5	8.4
14	20.0	33.0	25.3	24.3	9.1	12.9	10.5	11.7
15	18.3	24.4	28.8	25.6	10.3	18.7	9.6	13.3
16	20.9	26.3	30.0	24.4	12.3	16.8	8.9	9.9
17	21.4	22.0	-	-	11.2	16.6	7.8	10.9
18	23.1	22.1	26.1	23.4	10.3	15.6	14.7	8.4
19	22.4	20.6	24.3	22.5	9.7	13.8	15.5	13.0
20	23.6	21.4	24.3	22.5	9.1	-	14.2	13.2
21	23.4	20.1	26.1	21.5	9.1	15.0	12.0	10.6
22	17.3	19.6	26.7	20.3	8.7	16.6	11.1	9.9
23	19.4	26.9	25.7	24.7	7.9	17.6	9.9	8.8
24	20.5	28.3	26.2	25.6	7.8	16.7	12.0	8.8
25	21.8	34.2	26.5	25.0	10.0	13.5	17.3	7.8
26	20.0	32.3	24.7	20.7	10.7	18.1	16.5	7.3
27	21.0	28.1	25.2	21.2	11.4	17.5	14.2	7.9
28	19.7	24.3	26.5	22.5	9.8	16.5	13.1	11.0
29	21.8	31.7	27.1	20.5	10.0	14.1	10.9	11.7
30	17.8	24.4	26.2	18.2	9.4	-	16.0	12.8
31	19.6	27.6	25.4	19.2	8.6	16.0	17.5	12.0
32	21.2	26.7	25.7	21.7	10.1	18.1	15.9	12.3
33	22.5	30.5	24.7	25.8	10.1	18.1	15.9	11.6
34	24.7	28.9	24.7	26.9	10.2	16.2	12.6	10.8
35	23.4	30.6	23.2	24.2	9.3	16.3	17.0	10.3
36	23.2	26.4	23.2	23.5	9.1	13.3	16.9	10.0

TABLE 8.9B: SERIAL LH DATA (u/l) FROM PATIENTS WITH PCOS SAMPLING IN EARLY FOLLICULAR PHASE OF THE CYCLE WHERE APPROPRIATE - SAMPLES 37-72

SUBJECT	1	2	3	4	5	6	7	8
37	19.1	26.4	28.2	23.5	-		16.1	10.0
38	20.5	26.4	28.2	25.2	9.0		12.1	9.9
39	25.4	24.6	27.9	28.0	8.5		11.1	9.2
40	20.4	28.6	26.6	27.2	9.5		12.3	8.3
41	22.3	38.5	24.9	-	9.9		15.2	11.7
42	21.6	28.9	24.9	-	10.2		15.5	11.8
43	-	32.2	26.0	-	9.0		13.0	11.5
44	23.0	31.5	29.0	-	7.6			8.8
45	19.8	39.0	29.4	-	8.8		12.2	10.7
46	22.4	28.4	31.0	-	7.8			8.8
47	23.6	31.5	29.0	-	7.6			8.0
48	21.0	26.5	26.2	-	8.5			
49	24.2	21.2	24.5	25.4	9.8			
50	24.0	25.4	25.4	-	10.4			
51	22.8	23.3	26.7	23.2	9.7			
52	24.5	48.3	27.5	21.6	9.6			
53	20.9	44.7	28.3	20.8	9.1			
54	22.8	57.8	27.7	21.2	8.2			
55	22.1	62.5	28.0	25.5	8.3			
56		61.6	27.8	27.7	8.1			
57		46.3	26.9	32.5	7.5			
58		35.2	25.1	28.9	7.0			
59		29.1	24.4	31.8	8.7			
60		30.5	23.5	27.2	9.7			
61		31.6	25.7		10.5			
62		23.9	24.8		9.8			
63		22.8	26.6		9.1			
64		21.9	26.5		9.0			
65		26.3	25.6		8.2			
66		40.8	25.0		8.5			
67		40.9	23.5		7.6			
68		48.1	21.8		6.7			
69		40.7	25.4		7.1			
70		26.6	28.2		9.9			
71		33.5	28.3		10.5			
72		30.6	27.8		10.0			